THE EFFECT OF AGING ON MYELINATING GENE EXPRESSION AND OLIGODENDROCYTE CELL DENSITIES

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By

Rubin Jiao

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Adil J. Nazarali
College of Pharmacy and Nutrition
University of Saskatchewan
110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

OR

J. Ronald Doucette
Department of Anatomy and Cell Biology
College of Medicine
University of Saskatchewan
Health Sciences Building
Saskatoon, Saskatchewan S7N 5E5
Canada

ABSTRACT

During aging, there is a decrease both in the stability of central nervous system (CNS) myelin once formed and in the efficiency of its repair by oligodendrocytes (OLs). To study CNS remyelination during aging, I used the cuprizone (a copper chelator) mouse model. Inclusion of cuprizone in the diet kills mature OLs and demyelinates axons in the rostral corpus callosum (RCC) of mice, which enabled me to characterize age-related changes (i.e., 2-16 months of age) in glial cell response during the recruitment (i.e., demyelination) and differentiation (i.e., remyelination) phases of myelin repair. I found that the time between 12 and 16 months of age is a critical period during which there is an age-related decrease in the number of OL lineage cells ($Olig2^{Nuc}$+ve/GFAP-ve cells) in the RCC of both control mice and mice recovering from cuprizone-induced demyelination. My results also show there was an age-related impaired recruitment of progenitor cells to replace lost OLs even though there was no major age-related decrease in the size of the progenitor cell pool (PDGF α R+ve/GFAP-ve, and $Olig2^{Nuc}$+ve/PDGFαR+ve cells). However, there were cuprizone-induced increased numbers of astrocyte progenitor cells ($Olig2^{Cyto}$+ve/PDGFαR+ve) in these same mice; thus PDGFαR+ve progenitor cells in mice as old as 16 months of age retain the ability to differentiate into astrocytes, with this fate choice occurring following cytoplasmic translocation of Olig2. These data reveal for the first time age-related differences in the differentiation of PDGFαR+ve progenitor cells into OLs and astrocytes and lead me to suggest that during aging there must be a transcriptional switch mechanism in the progenitor cell fate choice in favour of astrocytes. This may at least partially explain the age-related decrease in efficiency of OL myelination and remyelination.

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This thesis is dedicated to my parents, my grandparents, and my wife. All of them inspired, supported, and encouraged me throughout my graduate study program. I sincerely appreciate. Particularly, I dedicate my thesis to my grandmother who passed on Sept. 16, 2009. It is a pity that you can not celebrate with us. I will always rememorize you when I see my thesis since I successfully defended on Sept. 16, 2010. This date means too much to me, I will never forget it. I miss you.

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LIST OF ABBREVIATIONS

3% SM-PBST	3% skim milk and 1% Triton X-100 in PBS
Aβ	Amyloid β-peptide
AD	Alzheimer's disease
bHLH	basic Helix-Loop-Helix
BBB	Blood-Brain Barrier
BDNF	Brain Derived Neurotrophic Factor
BMP	Bone Morphogenetic Protein
CC	Corpus Callosum
CGT	Ceramide Galactosyl Transferase
ChIP	Chromatin Immunoprecipitation
CNP	2',3'-cyclic nucleotide-3'-phosphodiesterase
CNS	Central Nervous System
E	Embryonic Day
EAE	Experimental Autoimmune Encephalomyelitis
EtBr	Ethidium Bromide
FGF	Fibroblast Growth Factor
GalC	Galactocerebroside
GCM	Glial Cells Missing
GFAP	Glial Fibrillary Acidic Protein
HDACs	Histone Deacetylases
IGF	Insulin-like Growth Factor
MAG	Myelin Associated Glycoprotein
MBP	Myelin Basic Protein
MHV	Mouse Hepatitis Virus
MOG	Myelin Oligodendrocyte Glycoprotein

MS	Multiple Sclerosis
NCAM	Neural Cell Adhesion Molecule
NFT	Neuronfibrillary Tangels
NGF	Nerve Growth Factor
NP	Neuritic Plaque
OB	Olfactory Bulb
OL(s)	Oligodendrocyte(s)
OPC(s)	Oligodendrocytes Precursor Cell(s)
PBS	Phosphate Buffered Saline
PDGFα	Platelet Derived Growth Factor alpha
PDGFαR	Platelet Derived Growth Factor alpha Receptor
PLP	Proteolipid Protein
pMN	Motor Neuron Progenitor Domain
PNS	Peripheral Nervous System
RCC	Rostral Corpus Callosum
RQ	Related Quantitative
RT	Reverse Transcription
SCP	Superior Cerebellar Peduncle
SFV	Semliki Forest Virus
SHH	Sonic Hedgehog
SVZ	Subventricular Zone
TH	Thyroid Hormone
TF	Transcription Factors
TMEV	Theiler's murine encephalomyelitis virus
VZ	Ventricular Zone
μm	micrometer

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I Hypothesis and Objectives

1.1 Hypothesis

There will be an age-related decline in the extent of remyelination by oligodendrocytes and in the expression of PDGF α R, MOG, Olig2, Nkx2.2, and CGT genes after cuprizone treatment.

1.2 Objectives

1.2.1 To quantify the cell density of OL lineage cells after cuprizone treatment in mice of different ages

1.2.2 To quantify the expression levels of *PDGFαR*, *MOG*, *Olig2*, *Nkx2.2* and *CGT* after cuprizone treatment in mice of different ages

II Literature Review

2.1 Oligodendrogenesis

Astrocytes, microglia, and oligodendrocytes (OLs) comprise a group of cells known as the glial cells (Baumann and Pham-Dinh, 2001). The majority of the cells populating the vertebrate nervous system are glial cells and they play an important role in neuronal function and development. Glial cells have the capacity to respond to changes in both cellular and extracellular environments in the nervous system, which is vital for the maintenance of normal function of the nervous system (Barres, 2008; Lepore, *et al*., 2004). Improper participation of glial cells in the central nervous system (CNS) has been associated with brain dysfunction (Venancel, *et al*., 1997), and a considerable amount of research has been conducted to investigate the relationship between glial cells and pathology of the CNS (Seqal, *et al.*, 2007; Morath, *et al.,* 2001; Breteler, *et al.*, 1992).

Oligodendrocytes (OLs) are myelin forming cells in the vertebrate CNS (Baumann and Pham-Dinh, 2001). Most OL cell clusters are located in the white matter of the CNS where they initially originate from highly mitotic and migratory oligodendrocyte precursor cells (OPCs) (Warf, *et al*., 1991; Levison, *et al*., 2003). During development in later embryonic and early postnatal periods, OPCs can migrate and differentiate into mature OLs. Once mature, OL processes will connect and wrap around axons, eventually forming the myelin sheath (Inoue, *et al*., 1996; Connor and Menzies, 1996). This process is defined as myelination. Myelination is extremely complex and the mechanism of how OLs wrap around axons has not been fully characterized. This literature review will provide a basic understanding of OPC development and a general description of the OL lineage cells, combined with the transcriptional factors affecting OPC differentiation and maturation. Furthermore, the current state of knowledge on myelination and remyelination, as well as the key components of myelin, will also be summarized. Finally, demyelination diseases and animal models to study remyelination will be reviewed.

2.1.1 Introduction to Glia

The two major cell constituents of the vertebrate brain are neurons (10%) and glia (90%) (Figure 1). Interestingly, some of these cells originate from different regions of the CNS. Neurons, macroglia including astrocytes, and OLs originally derive from the epithelial cells of the neural tube during early development (Morest and Silver, 2003). However, microglia are derived from the mesoderm (Kurz and Christ, 1998). Moreover, Schwann cells, located in the vertebrate peripheral nervous system (PNS) [that play similar roles to OLs in the CNS] myelinate PNS axons (Baumann and Pham-Dinh, 2001).

The most common roles of glial cells are communication with neurons and maintenance of the normal functioning of the nervous system. A functional relationship between neurons and glial cells, such as electrical insulator role of OLs and Schwann cells in vertebrate nervous system has been demonstrated (Baumann and Pham-Dinh, 2001). However, much of this knowledge is only beginning to be explored. The basic question to explore the function of glial cells is: How do glial cells communicate with neurons? Recent researches indicate that the synapse works as a key site of neuronal and glial interaction, in structural, biochemical and physiological aspects (Goslin and Banker, 1990; Keyser and Pellmar, 1997; Chisari, *et al.*, 2010). Researchers have used co-culture technology to show certain effects of glial cells. For example, glial cells are found to have an effect on synaptogenesis and synaptic function (Pfrieger and Barres, 1997).

Moreover, glial cells including astrocytes, OLs, and Schwann cells have many ion channels. Glial cells express all major types of ion channels such as K^+, Na^+, and Ca^+ (Chittajallu, *et al.*, 2004). These glial cells express similar ion channels to those found in neurons (Baumann and Pham-Dinh, 2001); prior to this discovery neuron were considered to be the only cell type that produced electric impulses in the brain. However, recent findings suggest that OL lineage cells have the potentials to fire electrical impulses (Karadottir *et al.*, 2008). Due to the various roles and electrical potential, details of diverse glial cells will be reviewed in the following paragraphs.

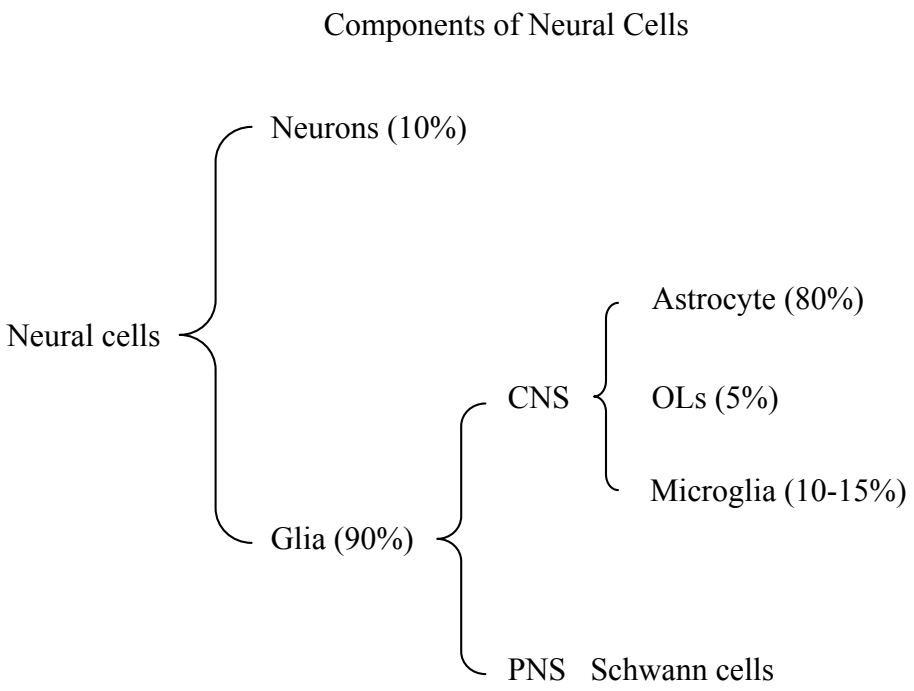


Figure 1. Components of Neural Cells. In the vertebrate nervous system, neural cells include neurons (10%) and glial cells (90%). Astrocytes (80%), OLs (5%) and microglia (10-15%) are three major cell types that maintain normal function of the CNS (Baumann and Pham-Dinh, 2001). In the PNS, Schwann cells rather than OLs form myelin sheaths.

2.1.1.1 Astrocyte

Astrocytes are the most abundant cells in the CNS. The generation of astrocytes begins in late embryonic stage, and continues during neonatal and postnatal period (Wang and Bordey, 2008). Glial fibrillary acidic protein (GFAP) is commonly expressed in astrocytes and is an important marker of these cells. However, only a small number of astrocytes are GFAP+ve in the cortex and hippocampus (Giffard and Swanson, 2005). The different lineages of astrocytes suggest that they may have different functions. Astrocytes can also function as stem cells in the nervous system (Guillemot, 2007). Researchers in the last decade have indicated that GFAP-expressing cells can also contribute to the cell regeneration. For example, the multipotent neural stem cells in adult subventricular zone (SVZ) were detected to express GFAP (Baumann and Pham-Dinh, 2001; Doetsch, et al., 1997). These stem cells were suggested to belong to the astrocyte family since they had GFAP-expressing properties. Moreover, in the CNS, astrocyte precursors can also undergo either gliogenesis or neurogenesis (Guillemot, 2007). This dual-directionality of cell development is not uncommon in brain formation. For example, OPCs can differentiate into either OLs or astrocytes during adult gliogenesis under different conditions (Zhao, *et al*., 2009). Recent finding also indicates astrocyte precursors have the ability to form neurons (Rivers, *et al.,* 2008). The switch between the two cell types is regulated by a cascade of transcription factors (TFs) (Nicolay, *et al.*, 2007; Islam, et al., 2009; Dimou, et al., 2008; Tatsumi, et al., 2008).

In the past, astrocytes were considered as "brain glue" because they lacked the ability to form action potentials. However, they are much more important because they promote neuronal maturation, neuronal survival and synapse formation during the development (Wang and Bordey, 2008). Commonly, an important role of astrocytes is to produce intermediate filaments that form the cytoskeleton in the brain, which further maintains the proper environment for the CNS (Pekny, *et al.*, 2007). In addition, astrocytes are well-known to release growth factors including nerve growth factor (NGF),

brain derived neurotrophic factor (BDNF) and fibroblast growth factor (FGF). These factors control the maturation and survival of neuronal cells. Another important role of astrocytes is contributing to blood-brain barrier (BBB). Researches indicate that BBB tight junction proteins are up-regulated by co-culture with astrocytes (Wolburg, *et al.*, 2009; McQuaid, *et al.*, 2009). It is believed that astrocytes can also maintain microvascular permeability in adult brain (Kaur, et al., 2008). Also, astrocytes are helpful to metabolism. It is well established that astrocytes metabolize glutamate into glutamine via glutamine synthetase (Albrecht, *et al.*, 2007); moreover, since glutamate transporter is essential to the proper neuronal activity, astrocytes support the metabolic need to the nervous system.

Interestingly, astrocytes and OLs keep a tight relationship *in vivo* and *in vitro (*Nait-Oumesmar, *et al.*, 2008; Ihrie and Alvarez-Buylla, 2008*)*. Several pubications show a lineage relationship between these two types of cells (Nait-Oumesmar, *et al.*, 2008; Ihrie and Alvarez-Buylla, 2008). For example, GFAP expression has been observed in immature OLs (Dawson, *et al.*, 2000); co-expression of GFAP in conjunction with myelin-antigens has also been found in mouse brains (Dawson, *et al.*, 2000). Also a dysfunction in astrocytes can induce serious disorders, such as stroke and cerebral ischemia (Kahle, et al., 2009; Lindsberg, et al., 2010). However, the details of the various roles of astrocytes are not clearly defined.

2.1.1.2 Oligodendrocytes

OLs can also participate in the formation of nodes of Ranvier, which play an important role in fast saltatory transduction (Wolswijk and Noble, 1989). Depending on the morphology and localization, OLs are classified into four subtypes (Baumann and Pham-Dinh, 2001). In most aspects, type I and type II OLs are very similar. However, type I OLs mainly exist in the brain and spinal cord, whereas type II OLs are only observed in the white matter. Compared to type I and type II OLs, type III OLs have larger cell bodies and fewer processes, and typically exist in the cerebral and cerebellar

peduncles. Type IV OLs do not have processes, but can form long myelin sheaths around axons directly by the cell body (Wolswijk and Noble, 1989). All four types of OLs can form myelin sheaths even though the number of their processes is significantly different. In addition, a small population of OLs in the CNS do not have the capacity to myelinate axons, and are termed "satellite OLs" (Ludwin, 1979).

During OL development and maturation, many factors are necessary. These factors have multiple effects in OLs proliferation, differentiation, and maturation. For example, platelet derived growth factor (PDGF) is synthesized by both astrocytes and neurons during development (Kwon, 2002). PDGF is a survival factor for OPC, and is a mitogen for progenitors (Baumann and Pham-Dinh, 2001). Basic FGF is another mitogen for OL progenitors. It up-regulates the expression of platelet derived growth factor alpha receptor (PDGFαR), further increases the opportunity for OL progenitors to respond to PDGF (Redwine, et al., 1997). In addition, insulin-like growth factor (IGF) plays an important role in stimulating OL proliferation (Ye, et al., 2010) as well as acting as a potent survival factor for OLs (Ye, et al., 2010). In IGF-transgenic mice, the percentage of myelinated axons is significantly increased, and the myelin sheaths are thicker than control mice (Baumann and Pham-Dinh, 2001).

The main function of OLs is to form myelin sheaths around axons. Significant research has demonstrated the important role of OLs in both development and adult CNS (Zeng and Jung, 2008; Schnaar and Lopez, 2009; Baumann, *et al*., 2001). Myelin is critical to saltatory transduction, thus the dysfunction of OLs can lead to myelin sheath destruction and to demyelinating diseases such Multiple Sclerosis (MS) but also can induce mental disorders (Noble, 2004; Seqal, *et al.*, 2007). The relationship between OLs and myelin development will be demonstrated in subsequent chapter that will follow.

2.1.1.3 Microglia

Unlike macroglia cells, microglia are derived from myelomonocytic cells, which originate from the mesoderm of the embryo (Kurz and Christ, 1998). During

development, the precursors of microglia undergo proliferation, migration, and differentiation, finally settling in the CNS to function as gatekeepers in the CNS (Kaur, *et al.*, 2010). Research indicates that amoeboid microglia are capable of working as phagocytes both *in vivo* and *in vitro* (Pivneva, 2008). In the normal brain, microglia remain in the resting stage and the cell body remains as a small soma. However, microglia shift to an activated state after brain injury. A fraction of the activated microglia consequently become phagocytic (Pivneva, 2008). Both activated microglia and phagocytes provide a defensive system in the brain to protect it from various injuries as well as diseases (Pivneva, 2008). In the past decade, many publications have reported on the close relationship between microglia and several disorders (Gandhi, *et al.*, 2010; Gay, 2007). For example, microglia have been found to participate in all phases of MS. In addition, it has been revealed to be involved in age-related neurodegenerative diseases, notably Alzheimer's Disease (AD). Many studies have shown s connection between activated microglia and amyloid plaques, which point out microglia play an important role in AD progression (Imbimbo, 2009; Shie, *et al.*, 2009).

Microglia are also essential for CNS development. For example, microglia can induce excess NGF-expressing neurons to undergo apoptosis, which regulates the quantity of neurons in the CNS (Harada and Harada, 2004). Hence, microglia play a crucial role in maintaining normal neuronal architecture by inducing overloaded NGF-expressing neurons to undergo apoptosis (Mizuno, *et al.*, 2004).

2.1.1.4 Schwann cell

Schwann cells perform a similar role in the PNS to that of OLs in the CNS. Schwann cells originate from Schwann cell precursors (Woodhoo and Sommer, 2008). The precursors are derived from neural crest cells. During vertebrate development, the precursor cells migrate and differentiate into mature Schwann cells that consequently myelinate axons in the PNS. Interestingly, myelinating Schwann cells can only myelinate axons with a 1:1 ratio. Hence, one Schwann cell only myelinates one axon (Baumann and

Pham-Dinh, 2001). The underlying mechanisms by which Schwann cells myelinate axons are not clear, but OLs and Schwann cells are proposed to share some factors involved in myelination (Ogata, *et al.*, 2006; Rajasekharan, 2008).

2.1.2 Stepwise development of oligodendrocyte lineage cells

Improvements in immunohistochemical techniques combined with developments in molecular biology have widely expanded our knowledge regarding OL development and lineage. Observations from *in vivo* and *in vitro* experiments show a linear progression of stages during OL development (Woodruff, *et al.*, 2001; Zhang, 2001; Miller, 2002; Raff, *et al.*, 1984). The identification of various cellular markers that determine cell fate and maturation also reveal a stepwise development of oligodendrocyte lineage cells (Pfeiffer, *et al.*, 1993; Raff, 1989; Baumann and Pham-Dinh, 2001; Le Bras, *et al.*, 2005).

2.1.2.1 Stepwise oligodendrogenesis

Details of OL development and maturation have been described in many models, and researchers are working towards a system that represents all OL lineage cells. However, it is unlikely that all details of OL development will be the same in different regions of the CNS. Cells are generated at different times due to anatomical variations in different regions. For example, the majority of the white matter in the brain is centrally located while most white matter in the spinal cord is located in the periphery. Despite the differences mentioned above, all glial cells share significant similarities during their generation. First, most cells in the CNS including neurons originate from the ventricular zone (VZ) in the embryonic stage (Hardy and Friedrich, 1996; Vallstedt, *et al.,* 2005; Le Bras, *et al.*, 2005), and a variety of transcriptional factors contribute to their developmental progress (Nicolay, *et al*., 2007). Immature OLs, which eventually become mature OLs, are located mainly in ventral regions of the neuraxis (Hardy and Friedrich, 1996). The fact that glial cells and neurons originate from the same precursors is well known; however, when the stem cells begin to differentiate and how this process is controlled remain the subject of ongoing research.

In general, OL development throughout the CNS follows a similar pattern. After neuronal stem cells divide into different precursor cells, the OL lineage division become A2B5+ve bipolar OPCs during the late embryonic phase (Hardy and Friedrich, 1996; Vallstedt, *et al.*, 2005), which can consequently differentiate into both OLs and astrocytes (Raff, *et al.*, 1984). OPCs migrate extensively throughout the CNS. This process is highly regulated by a large number of important molecules (Byravan, *et al*., 1994; Dai, *et al.*, 1997; Kuhn, *et al.*, 1999; Goldberg, *et al.*, 2004). After their migration and proliferation in the CNS, progenitors grow fiber tracts that consequently form white matter, and eventually transform into O4+ve pre-myelinating OLs (Pfeiffer, *et al.*, 1993). At this stage, they lose their mitogens, such as PDGFα, and thus stop proliferating. At the same time, these OPCs begin to differentiate into myelin basic protein (MBP)+ ve mature OLs (Figure 2) (Baumann and Pham-Dinh, 2001). Although they follow the same developmental patterns, variations exist between OL lineages in different regions in the brain, even in various lineages in the forebrain (Vallstedt, *et al.*, 2005; Nait-Oumesmar, *et al.*, 2008).

Some OLs and astrocytes share the same progenitors in the embryonic stage, particularly when they are generated from the SVZ (Nait-Oumesmar, *et al.*, 2008; Ihrie and Alvarez-Buylla, 2008). Studies indicate OLs in the Ventricular Zone (VZ) are generated during the first week of life in rodents, which is consistent with astrocytes (Kagawa, 2001; Baumann and Pham-Dinh, 2001). The earliest cells that express early markers of OLs have been found in the ventral forebrain. The progenitors begin to express DM-20 (Dickinson, *et al.,* 1996) and PDGFαR (Pringle and Richardson, 1993), which are markers of OPCs. Interestingly, markers for DM-20+ve and PDGFαR+ve do not appear in the same cell, though the relationship between these two types of cell markers is still unclear; these two cell lineages are proposed to divide into two classes of OLs (Pringle and Richardson, 1993, 2000; Spassky, *et al.*, 2000).

Both DM-20+ve and PDGFαR+ve cells appear in dorsal rather than ventral locations (Warf, *et al.*, 1991; Pringle and Richardson, 1993; Fok-Seang and Miller, 1994; Tekki-Kessaris, *et al*., 2001). These findings are further confirmed by observations that OPCs migrate extensively in a ventral to dorsal direction during the late embryonic period. During this period, SVZ cells do not express early markers of both OLs and astrocytes within the SVZ; instead, they begin to express early antigens for glial cells such as PDGFαR and NG2 after they exit the SVZ (Zhao, *et al.*, 2009). This dorsolateral SVZ provides cells that travel to the cortex, white matter, striatum, and olfactory bulb for both OL and astrocyte generation (Young, *et al.,* 2007).

OPCs are generated from the SVZ at the base of the third ventricle and migrate along the optic nerves (Sugimoto, *et al.*, 2001). However, this cell type shows a bipotential nature. The OPCs can develop into OLs or astrocytes due to the existence of the optic nerve and other factors (Calver, *et al*., 1998; Gross, *et al.*, 1996). Interestingly, OL development is not exactly the same even in the forebrain. For example, glial cell development in the optic nerves is delayed compared to OLs in the white matter. Also, these two cell types do not share the same progenitors. Optic nerve astrocytes appear to be more intrinsic, and likely arise from the radial glial cells that originate from the optic nerve neuroepithelium (Sugimoto, *et al.*, 2001).

In addition to the forebrain, another area that holds a large area of white matter is the cerebellum. The cerebellum contains OLs as well as astrocytes. Both these glial cells arise from the base of the cerebellum and share the same progenitor (Levine, *et al.*, 1993). During prenatal development, the progenitors migrate through the white matter tracts and form a giant area of white matter in radial directions (Levine, *et al*., 1993). Concurrently, some progenitors begin to express early markers, such as NG2 for OLs and GFAP for astrocytes. In addition, OL development in the spinal cord is highly dependent on dorsoventral patterning. Cells in the motor neuron progenitor domain (pMN) express the

basic helix-loop-helix (bHLH) transcriptional factor Olig2 (Fu, *et al.*, 2002; Novitch, *et al*., 2001).

Unfortunately, the mechanism that determines whether neuronal progenitor OPCs develop into OLs or astrocytes is still unclear. However, current research suggests a series of transcriptional factors may be involved in the process. For instance, results from different laboratories suggest expression of Olig2 switches the fate of progenitors and triggers the development of the OL lineage cells rather than astrocytes (Islam, *et al*., 2009; Dimou, *et al.*, 2008; Tatsumi, *et al.*, 2008). Information about these TFs will be discussed in a later section (Section 2.1.4).

2.1.2.2 Oligodendrogenesis in the adult CNS

Research in the past several decades suggests neurogenesis also occurs and is important for the normal development of the adult CNS (Fujimoto, *et al.*, 2009; Lee and Son, 2009; Ahlenius, *et al.*, 2009). Studies have demonstrated both neuron and glial cell proliferation in adult brain, and have broadened knowledge in this area.

Studies conducted by different research groups suggest glial cells may arise from either existing proliferating cells or from neuronal progenitors. However, increasing evidence support the latter theory (Ahlgren, *et al*., 1997; Kiernan and Ffrench-Constant, 1993; Almazan and McKay, 1992). Progenitors isolated directly from the adult CNS express several markers such A2B5, O4, and GFAP. In addition, an *in vivo* study found NG2 chondroitin sulfate proteoglycan positive cells in adult brain (Baracskay, *et al.*, 2007; Gard and Pfeiffer, 1990; McCombe, 2008). Though NG2 is found during oligodendrogenesis, it is now unclear whether NG2 is a marker for a single type or multiple types of glial cells. Many NG2+ve cells contact the node of Ranvier in white matter and synapses in gray matter, suggesting they may play a role in the maintenance of stable glial populations (Butt, *et al*., 2004).

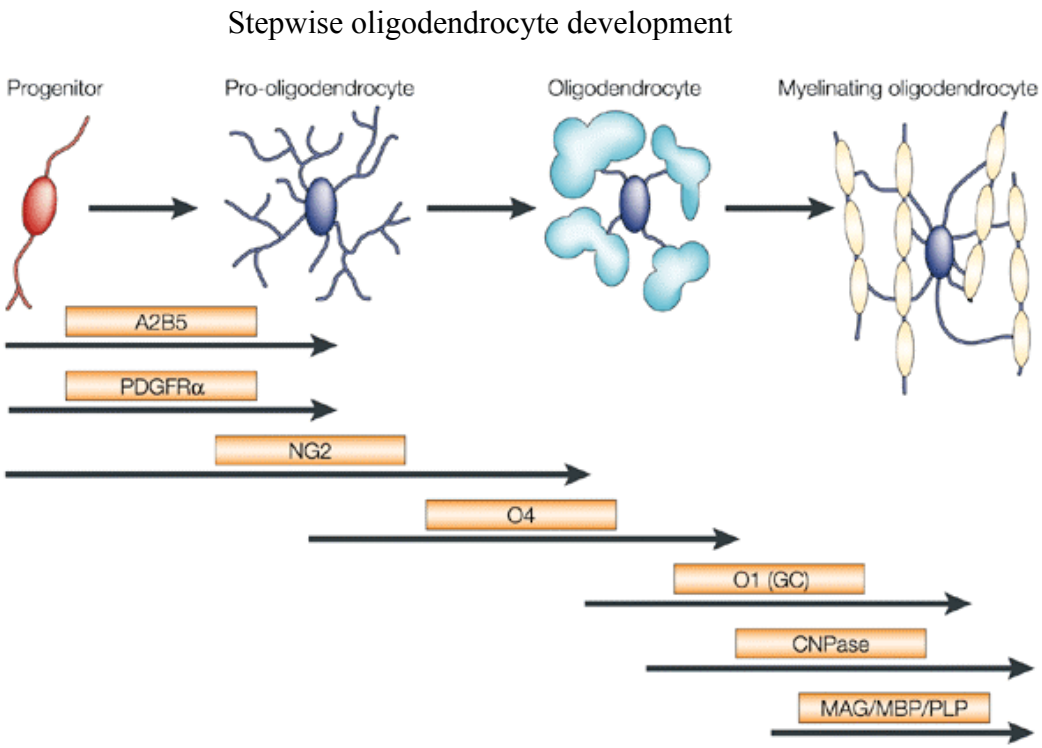


Figure 2. Stepwise oligodendrocyte development. OLs originate from bipolar progenitors. During their development, OPCs undergo A2B5+, NG2+, and O4+ stages before eventually becoming mature OLs. MBP and MOG are two major markers for OL maturation (taken from Zhang, 2001 with permission).

Moreover, studies in rodents and human indicate NG2 exists in adult brains in various pathologies, suggesting a small population of neuronal cells in adult brains may indeed maintain the ability to proliferate (Zhao, 2009; Komitova, *et al*., 2009).

2.1.3 Oligodendrocyte precursor cells

During development of the CNS, the mechanism determining the fate of the precursors of both neurons and glial cells is unknown. The gene that encodes 'glial cells missing' (gcm) protein induces the stem cells to lose their glial cell properties and switch into neurons (Ho, *et al.*, 2009; Soustelle and Giangrande, 2007; Soustelle, *et al.*, 2008). As mentioned above, both neurons and glial cells in the mammalian cortex originate from proliferating neuroepithelial cells in the VZ and SVZ. The growth factors and transcriptional factors circulating in the SVZ influence cell survival and are involved in proliferation and differentiation of the neuroepithelial cells. However, the focus on this project is on the OPCs arising from the multi-potential stem cells.

2.1.3.1 Development of OPCs during embryonic stages

OPCs originate from specific regions in the CNS. Two distinguishable populations of OPCs in the CNS come from the VZ and SVZ, respectively (Juliet, *et al.*, 2009; McMahon and McDermott, 2001; Nait-Oumesmar, *et al*., 2008). Although most OLs locate in the white matter, OPCs originate from the VZ and SVZ at a very early stage in the embryo.

The spinal cord is one of the systems studied in which OPCs have originated from VZ at early embryo stages. This system has been extensively studied and is well understood. In the spinal cord, the OPCs initially arise in the neural tube. At embryonic day 14 (E14) in rodents, OPCs begin to migrate in the ventral to dorsal direction and have the capacity to differentiate into OLs in the ventral region of the spinal cord (Timsit, *et al.*, 1995; Hardy and Friedrich, 1996). As mentioned before, two major markers of OPCs, DM-20 and PDGFαR, are never expressed together. However, either DM-20 positive or PDGFαR positive OPCs can eventually become mature OLs in the spinal cord.

The initial localization of OPCs in the ventral regions of the neural tube does not determine the localization of mature OLs. Mature OLs are localized not only in the spinal cord but also in the midbrain and forebrain (Ono, *et al.*, 2009; Fu, *et al*., 2003). In addition, oligodendrogenesis occurs in similar regions in the vertebrate CNS across different species including human, mouse, and chicken (Ono, *et al.*, 1997).

In addition to the OPCs in the VZ, another population of OPCs arises from the SVZ. The SVZ is a matrix that first appears during the later stages of embryonic development (Doetsch, *et al.*, 1997). It begins its extension five days after conception, at the peak of oligodendrogenesis (Luskin, 1988; Price and Thurlow, 1988). Although the majority of OLs come from homogeneous progeny, both OLs and astrocytes can also originate from precursor cells in the SVZ. In the SVZ of adult brain, some cells can even grow neurons and other glial cells (Rivers, *et al*. 2008). While some have assumed all cells in this structure are radial cells (Rakic, 1995), several studies using various techniques suggest cells diffuse postnatally and even during embryonic development. A large population of OLs arises from the SVZ in the cerebrum and optic nerves of newborn rodents (Fernandez, *et al.*, 2004; Levine, *et al.*, 1993). The OPCs migrate and proliferate quickly and do not concurrently differentiate, which helps to maintain the correct function of mature OLs.

2.1.3.2 OPCs in adult CNS

OLs have the ability to myelinate axons, which is critical for proper repair of the CNS. In the human brain, OPCs can be recruited continuously and further differentiate into mature OLs and myelinate axons into the fifth decade of life (Bartzokis, 2004; Bartzokis, *et al.*, 2004). Thus, adult OPCs play important roles not only with respect to injury repair but also for normal function of myelination.

Recent research has demonstrated not all OPCs differentiate during CNS development (Figure 3). Some OPCs in the adult CNS still have the ability to differentiate. Differences between the OPC differentiation during development and in the

adult CNS include antigenic phenotype and proliferation rate. In the adult CNS, the activity of OPCs is relatively low due to the lower expression level of mitogens, which limit the proliferation rate. Under normal conditions, adult OPCs are inactive, but once mature OLs die or undergo apoptosis, the OPCs are rapidly recruited and consequently undergo differentiation and maturation.

2.1.4 Transcriptional control of oligodendrogenesis

During the formation of the myelin sheath, OLs undergo complicated regulatory mechanisms that are not fully understood. The specification and maturation of OLs are, for the most part regulated by multiple transcriptional factors (TFs). The majority of these TFs are regulated by signaling pathways such as sonic hedgehog (Shh), bone morphogenetic protein (BMP), and Notch (Nicolay, *et al.*, 2007). Interestingly, these signaling pathways and TFs play multiple roles in OLs regulation, as either inductive, inhibitory, or permissive factors (Figure 4).

Adult neural genesis in the CNS

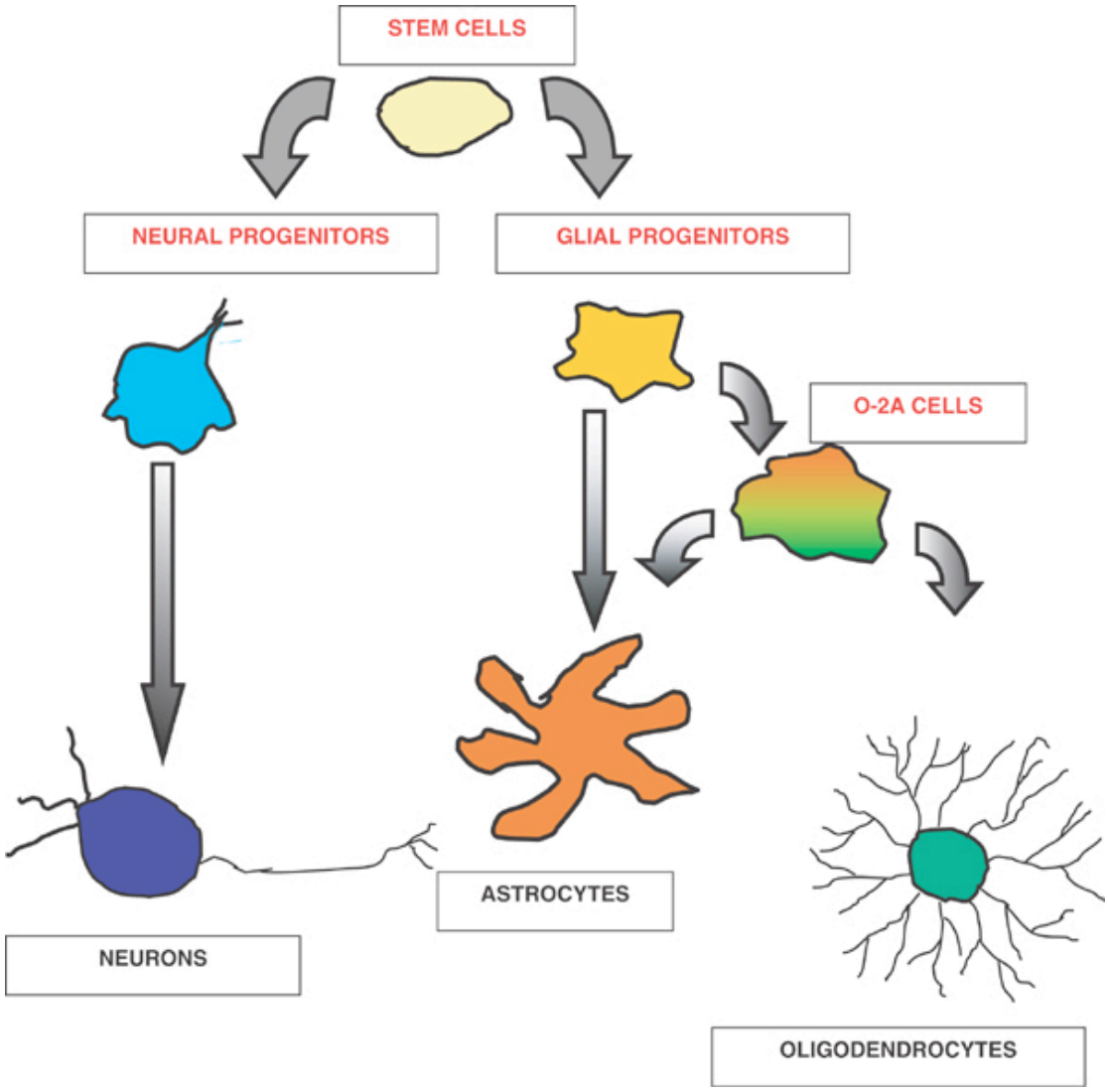


Figure 3. Adult neural genesis in the CNS. Neural stem cells exist in the adult brain, and retain the ability to differentiate into either neurons or glial cells, including astrocytes and OLs (taken from Galderisi, *et al.*, 2003 with permission).

Basic transcriptional network of OL development

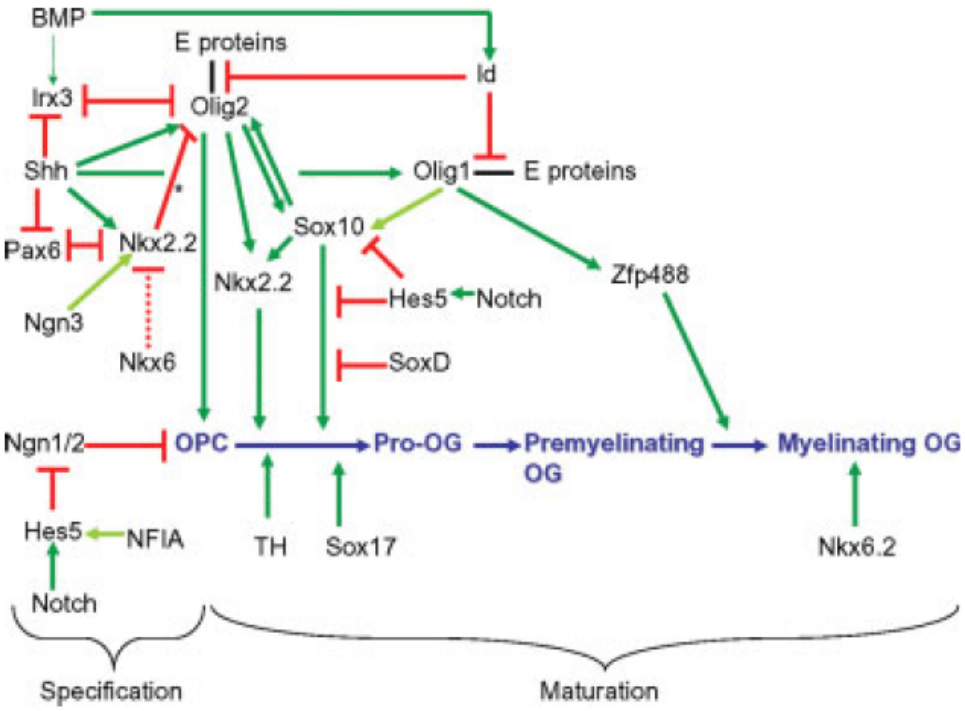


Figure 4. Basic transcriptional network of OL development. Multiple TFs control OL development. Expression levels of TFs at different times determine the cell fate and further regulate OL maturation (taken from Nicolay, *et al.*, 2007 with permission).

2.1.4.1 Inductive factors to OLs specification

PDGFαR is essential during the early development of OLs in the pMN domain, which is characterized by the bHLH oligodendrocyte specific TFs, Olig1 and Olig2 (Lu, *et al.*, 2000). However, in the absence of Olig2, PDGFαR is expressed at a lower level with no expression in the spinal cord (Lu, *et al*. 2002). Expression of Olig1 and Olig2 are affected by the Shh signaling pathway, which is concentration dependent (Lu, *et al.*, 2000). Recent research demonstrates the consistent role of the Shh signaling pathway throughout CNS development. For example, Shh mutant mice lack Olig1, Olig2, and PDGFαR expression (Lu, *et al.*, 2000; Alberta, *et al.*, 2001). Conversely, higher expression levels of Olig2 and PDGFαR are found when a Shh-expressing retrovirus is injected into the embryo (Nery, *et al.*, 2001). However, the function of Shh varies in different areas throughout the CNS. Although Olig2 plays a critical role in the induction of PDGFαR expression in the spinal cord, PDGFαR is still expressed in the forebrain and hindbrain when Olig2 is absent (Lu, *et al.*, 2000). Interestingly, PDGFαR expression is absent in Olig1/Olig2 knockout mice (Lu, *et al*., 2000; Alberta, *et al.*, 2001).

2.1.4.2 Signaling pathways involved in development of OLs

Three additional TFs—Nkx2.2, Pax6, and Irx3—together with Olig2 define the boundaries of the pMN domain (Zhou, *et al.*, 2000; Briscoe, *et al.*, 2000). Specifically, Nkx2.2 and Pax6 demarcate the ventral pMN, whereas Olig2 and Irx3 define the dorsal pMN (Zhou, *et al.*, 2000; Briscoe, *et al.*, 2000; Novitch, *et al.*, 2001). Because the TFs control the boundaries of the pMN domain, the expression of particular TFs in certain areas modulates the temporal development. For example, a one-day delay in PDGFαR expression has been discovered in Pax6 deficient mice (Briscoe, *et al.*, 2000). In addition, Nkx2.2-repressed Olig2 expression has been demonstrated in early development (Zhou, *et al.*, 2001; Novitch, *et al*., 2001). Furthermore, progress may be delayed by Irx3-suppressed Olig2 expression though the precise function of Irx3 in OL development has not yet been determined (Novitch, *et al.*, 2001).

Unlike Shh signaling pathways that promote differentiation of OL lineage cells, BMP regulates TFs that suspend OL development (Vallstedt, *et al.*, 2005). Recent research indicates blocked BMP signaling results in an expansion of O4 positive cells in the embryo, suggesting the conversion of OL lineage cells (Mabie, *et al.*, 1999). Indeed, BMP suppress the expression of Olig2 in early development by enhancing Irx3 expression (Meyer and Roelink, 2003). In addition, BMP signaling may prevent OL maturation by enhancing Id2 and Id4 expression. Recent research demonstrates over-expression of Id2 and Id4 can prevent OPC differentiation into the GalC+ve stage of OL maturation (Samanta and Kessler, 2004; Wang, *et al.*, 2001; Kondo and Raff, 2000). Collectively, BMP signaling inhibits OL development.

Another essential signaling pathway for OL development is Notch signaling, which controls the neural-glial switch. Research indicates Notch represses the expression of Ngn1/2, which is necessary to determine the fate of OPCs. Notch signaling offers the environment for OLs specification (Zhou, *et al.*, 2001). Aside from its contribution in the specification of OL lineage cells, Notch signaling also prevents the maturation of OL lineage cells. Over-expression of Notch signaling has been associated with the accumulation of OPCs (Park and Appel, 2003). Furthermore, OPCs fail to differentiate when Hes5, the downstream target of Notch, is over-expressed (Deneen, *et al.*, 2006; Wang, *et al.*, 1998). On the other hand, a deficiency of Notch results in a higher expression level of MBP and proteolipid protein (PLP), markers of differentiating and mature OLs, in different regions in the brain (Park and Appel, 2003; Genoud, *et al.*, 2002).

2.1.4.3 TFs in OLs maturation

As discussed above, Shh and Notch signaling directly or indirectly stimulates the specification of OPCs. Conversely, BMP only negatively regulates OL specification. Under the control of these signaling pathways, TFs undergo a complex mechanism to regulate oligodendrogenesis. For example, Irx3 and Nkx2.2 affect the normal expression

level of Olig2 that is necessary for early OL specification. Hes5 can bind Sox10 and/or Mash1 and thus inhibit the maturation of OLs (Liu, *et al.*, 2006). Hes5 can also bind to the MBP promoter without affecting its activity, which suggests an indirect regulation of OL maturation (Liu, *et al.,* 2006). Olig1 promotes OL maturation via Sox10 and Zfp488. In the absence of Olig1, Zfp488 does not express in the forebrain, cerebellum, and spinal cord, which consequently leads to a reduction of MBP (Wang, *et al.*, 2006). Sox17 is another important TF for OL maturation that can decrease cell proliferation while enhancing MBP transcription (Sohn, *et al.*, 2006). Nkx6.2 is required during myelination and has similar expression profile to MBP and PLP, which are the main components of myelin sheaths (Awatramani, *et al*., 1997). Another important TF that regulates OL development is MyTI. When MyTI is induced, OL maturation is enhanced (Nielsen, *et al.*, 2004). Conversely, the truncation of MyTI results in a decline in OL differentiation (Nielsen, *et al.*, 2004). Additionally the homeobox (Hox) TF family may also regulate myelination in the CNS. Hoxd1 can bind to the promoter region of the myelin oligodendrocyte glycoprotein (MOG) gene (Booth, *et al.*, 2007), which plays a critical role in the final step of myelin sheath formation. Hoxd1 belongs to the Hox gene family, which is critical during embryo development. The expression profiles of Hoxa2, Hoxb3, and Hoxb4 during OL development have been recently published (Hao, *et al*., 1999; Nicolay, *et al.*, 2004; Nicolay, *et al.*, 2004b).

In addition to TFs, thyroid hormone (TH) also plays an important role in OL development. TH receptors are identified in both OPCs and OLs (Fernandez, *et al.*, 2004; Gao, *et al.*, 1998; Barres, *et al.*, 1994). In fact, murine hypothyroidism facilitates the proliferation of NG2-expressing cells in the SVZ. Interestingly, TH has different effects on the development of OLs. For example, purified murine OPCs from E7 stop dividing and rapidly begin to differentiate when exposed to T3, while those from E18 remain in cell cycle in the presence of T3 (Gao, *et al.*, 1998). Hence, TH activates the effector to influence stepwise OL maturation rather than indirectly triggering cell differentiation.

2.2 Introduction to myelination and remyelination

The main function of OLs is to generate the myelin sheath that insulate axons in the CNS; Schwann cells perform a similar role in the PNS. Myelin sheath is a fatty layer that insulates axons and facilitates saltatory conduction within neurons (Bauer, *et al*., 2009; Collarini, *et al.*, 1991; Lopes-Cardozo, *et al.*, 1989). Using the plastic coat around electrical wires as a comparison, myelin sheaths are in contrast composed of living tissue that need to be produced constantly throughout the lifespan by special myelinating cells—OLs in the CNS and Schwann cells in the PNS.

OLs and the axons around which the OLs form myelin sheaths exist interdependently. The proliferation and differentiation of myelinating OLs are regulated by a variety of signaling pathways. Also, the growth of myelin sheaths is determined by normal function and integrity of the axons (Kagawa, *et al.*, 1994; Readhead, *et al.*, 1994). It is important to maintain axons in the CNS for normal OL function. In some cases, loss of axons results in degeneration of the OLs and eventually leads to pathological abnormalities (Anderson, *et al*., 1998). Interestingly, if their structure is degenerated, myelin sheaths in the adult CNS can be reformed by myelinating OLs through the process of remyelination (Mi, *et al.*, 2008; Gallo and Armstrong, 2008; Nait-Oumesmar, *et al*., 2008).

2.2.1 Myelin

Myelin is an essential component of white matter in the vertebrate CNS. The main components of myelin are lipids and proteins that are generally found only in OLs (Bronstein, *et al.*, 1997; Coffey and McDermott, 1997). The lipids provide both the structure for the myelin sheaths as well as the ability to insulate the axons; the myelin proteins maintain the normal function of OLs during development and further assist myelin sheath formation. With recent knowledge regarding the localization and function of myelin proteins, details of OL development and myelination are becoming better understood.

The major lipids composing myelin are cholesterol, phospholipids, and glycolipids (Agrawal, *et al*., 1982; Tetzloff and Bizzozero, 1998; Baumann and Pham-Dinh, 2001). Most cholesterol is produced by OLs in the brain. It helps to compact myelin sheath formation due to its high hydrophobicity (Rosetti, *et al*., 2008). Cholesterol is also required during axon regeneration (Chakraborty, *et al.*, 1997). In addition, the synthesis of cholesterol is critical to myelination since an abnormal synthesis of cholesterol is strongly associated with neurological diseases such as Alzheimer's disease.

Another myelin component that has been widely studied is the glycosphingolipids, particularly galactocerebrosides (GalC) and its derivatives, sulphatides, which are used as markers following immunohistochemical analysis to identify OLs (Dupree, *et al.*, 1998; Matsubayashi, *et al.*, 2009). GalC is one of the early markers that reproducibly presents itself on the processes of mature OLs (Matsubayashi, *et al.*, 2009). Generally, GalC appears when pre-mature OLs lose the expression of A2B5 (Baumann and Pham-Dinh, 2001). Ceramide galactosyl transferase (CGT), the enzyme that catalyzes the final step of GalC synthesis, is used to test the function of GalC in OL development. Without the presence of GalC, myelin is not synthesized correctly. For example, in CGT knockout mice, saltatory conduction is blocked in the CNS, though OLs can still form myelin sheaths (Coetzee, *et al.*, 1996; Bosio, *et al.*, 1996). Furthermore, most CGT knockout mice die at the end of myelination period (Dupree, *et al.,* 1998). Interestingly, under electron microscopy, the abnormalities have only been shown in the CNS and not the PNS, indicating GalC is more critical to the proper myelin formation and axon-OL interaction in the CNS (Dupree, *et al.*, 1998).

As mentioned above, most myelin proteins are found only in myelin. The major components of myelin in the CNS are MBP and PLP. Myelin also contains other constituents including myelin associated glycoprotein (MAG), MOG, and 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNP) (Tetzloff and Bizzozero, 1998; Baumann and Pham-Dinh, 2001; Walsh and Murray, 1998). (Figure 5)

Structure of the myelin sheath

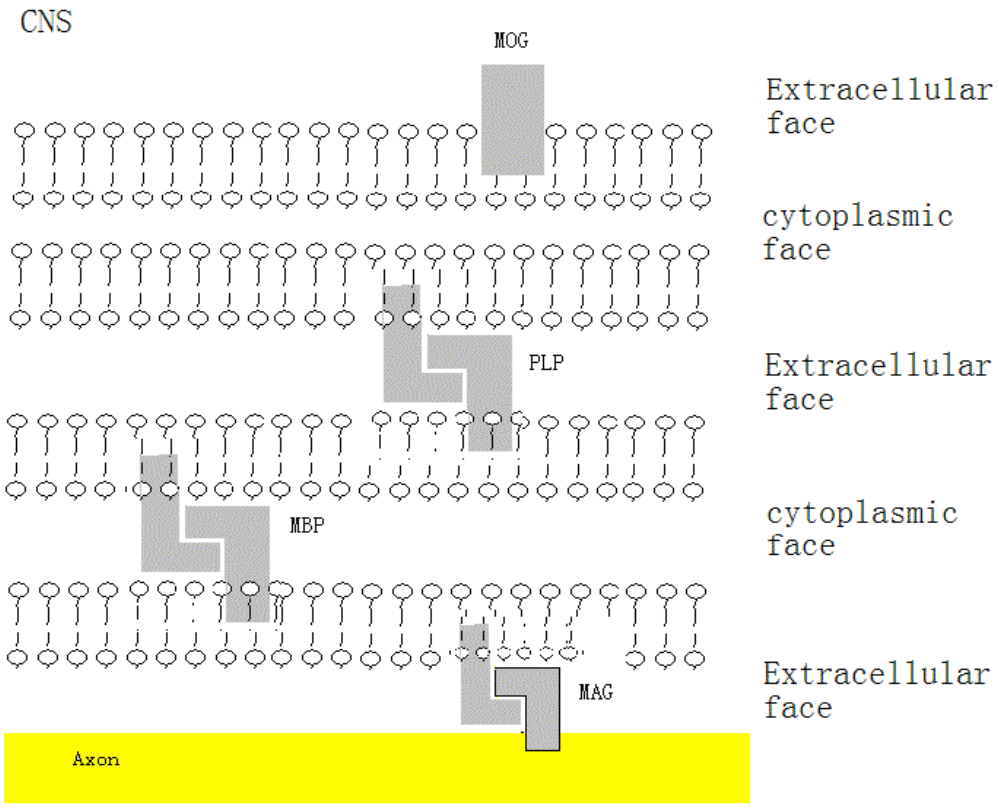


Figure 5. Structure of the myelin sheath. Within the myelin sheath, myelin proteins assist the formation of compact multi-layers. MOG, MBP, MAG, and PLP express during the later stages of myelination and act as markers of mature OLs (adapted from Verkharatsky and Butt, 2007).

PLP, the most abundant myelin protein in the CNS, constitutes up to 50% of myelin (Pham-Dinh, *et al.*, 1991). PLP has been studied for over 50 years, and its properties are well understood. In the CNS, both PLP and its isoform DM-20 are synthesized by OLs. A variety of functions of PLP have been proposed, such as axon-OL interaction, membrane adhesion, compact myelin sheath formation, and maintenance of axons. PLP knockout mice lose the ability to condense myelin sheaths as well as to maintain normal axonal activity (Klugmann, *et al*., 1997). In addition, research suggests PLP expression directly enhances the proliferation of OLs. As the major myelin proteins, PLP and DM-20 are also candidates for the study of autoimmune attack in demyelination diseases such as multiple sclerosis (MS) (Olson and Miller, 2009; Kooi, *et al.*, 2009). In the experimental autoimmune encephalomyelitis (EAE) model that mimics the pathology of MS, T-cells respond to PLP epitopes in the cytoplasm. Similarly, MS patients show increased levels of T-cells in their blood (Wang, *et al*., 2006). Thus, PLP plays important roles in both myelin formation and maintenance of normal axonal function.

MBP is the second most abundant protein in the CNS, and comprises 30% of the myelin proteins (Klugmann, *et al*., 1997). MBP has been found in both the CNS and PNS, and performs as a key factor in the fusion of the cytoplasmic interface between myelin layers. Research suggests MBP null mice lack compact myelin sheaths in the CNS; the MBP mutant rat shows a similar result (Kirschner and Ganser, 1980; Martini, *et al*. 1995). Multiple MBP isoforms have been confirmed in OLs and myelin sheaths, with humans and mice having 4 and 5 isoforms, respectively (Kimura, *et al.*, 1998; Kimura, *et al*., 1989). The main difference between these isoforms is the expression of exon 2 (Kimura, *et al*., 1989). Research indicates these isoforms are individually regulated and differently located, suggesting the proposed functions may be different (Kimura, *et al.*, 1998). In addition, MBP mediates the interactions with actin and tubulin. Studies suggest MBP causes G-actin polymerization, which further contributes to cytoskeleton formation in OLs (Boggs and Rangaraj, 2000).

MAG is one of the major components of glycoprotein in the CNS. MAG has both a membrane-spanning domain and extracellular region (Baumann and Pham-Dinh, 2001). MAG is proposed to interact with neural cell adhesion molecules (NCAM) during myelination. The functions of MAG have been studied in multiple knockout mice. Interestingly, the formation of myelin is almost normal in MAG-deficient mice but with a notable delay in myelin sheath compaction (Yin, *et al.*, 1998). MAG may also help OLs distinguish between myelinated and unmyelinated axons (Schachner and Bartsch, 2000). Moreover, MAG has been considered a key factor during the initial step of myelination. Indeed, both *in vivo* and *in vitro* studies demonstrate the association between MAG and axon-OL interaction. Another potential role of MAG is the inhibition of neurite outgrowths. For example, the abundant soluble extracellular domain of MAG released from myelin can inhibit the regeneration of axons (McKerracher, *et al*., 1994).

MOG is a glycoprotein that is found only within OLs in the CNS, and is a surface marker of mature OLs. MOG locates on the surface of membrane, and further appears on the most outside of the myelin lamellaes (Brunner, *et al.*, 1989). Thus, MOG is widely used as the final marker to indicate the maturation of OLs as it presents only in the late stages of myelination. Currently, MOG is considered as an adhesion molecule or cellular receptor that is involved in the completion and compaction of myelin sheaths (Coffey and McDemott, 1997). However, the precise function of MOG is unknown.

CNP is specific to both OLs in the CNS and Schwann cells in the PNS. It is expressed in the early stages of OL development. Two isoforms of CNP are distinguished: CNP1 and CNP2 (Walsh and Murray, 1998). Although the function of this enzyme has not been revealed, it has been suggested that CNP can regulate myelination. Recent studies indicate the dysfunction of CNP in the CNS can lead to unusual expansion of OL membranes (Gravel, *et al*., 1996; Nave and Trapp, 2008).

2.2.2 Myelination in developing CNS

Myelination of axons in the developing CNS includes a series of steps during which multiple TFs and surface molecules are required. Compared to Schwann cells that can only myelinate axons at a 1:1 ratio, mature OLs can myelinate multiple axons. On average, one OL can myelinate more than ten individual axons. However, multiple myelin sheaths around the same axon are rarely produced by an individual OL (Petters, *et al.*, 1991). Each myelin sheath is compacted with lamellae that extend from OL processes via cytoplasm that communicates with the OL cell body. In terms of the volume of myelin, OLs are the largest cells in the body. Once they become mature, OLs will contact and wrap the candidate axons, and consequently form the fatty myelin layers or lamellae. These processes are easily observed in the transverse section (Baumann and Pham-Dinh, 2001) (Figure 6).

The precise mechanisms involved in myelination have not yet been resolved. However, the entire process of myelination can be divided into three phases (Baumann and Pham-Dinh, 2001). In the first phase, OLs recognize candidate axons that require a myelin sheath. The pre-myelinating OLs can contact several axons, but only those beyond a critical diameter will be myelinated. In the second phase, myelinating OLs will wrap around the axons. Both adhesion and longitudinal outgrowths will be processed in this step. Although these adhesion molecules have not been identified, cell surface molecules such as MAG and NCAM likely play essential roles during this stage (Yin, *et al.*, 1998). After the initial outgrowth of myelin around the axon, subsequent steps occur in the third phase. First, non-compacted myelin lamellae interact within each other, and subsequently form compact myelin sheaths with multi-layers. Research indicates this step depends upon PLP and MBP. Without the presence of PLP, axon degeneration occurs, indicating the critical function of PLP during myelin sheath formation (Klugmann, *et al*., 1997; Kooi, *et al*., 2009). In addition, MBP-deficit mice lack a compact myelin structure (Kirschner and Ganser, 1980; Martini, *et al.,* 1995). As discussed above, myelin lipids

and proteins regulate correct special and temporal assembly of compact myelin sheaths during myelination.

The assembly of myelin sheaths is accompanied by formation of a bilayer of plasmalemma, maturation of axon-glial junctions, as well as arrangement of nodes of Ranvier (Schnaar and Lopez, 2009). In the CNS, nodes of Ranvier are assembled based on clusters of Na^+ (Zeng and Jung, 2008). Since Na^+ channels are present throughout the axons, the OL processes can recognize the signal and "grow" between Na^+ channel clusters, further from the unsheathed regions defined as the nodes of Ranvier. As myelin is found extensively in the vertebrate nervous system, this element is considered to be important in higher nervous function (Zeng and Jung, 2008). Indeed, both the high electrical resistance and low capacitance of myelin promotes signal impulse jumps from node to node. Rapid saltatory conduction results in faster signaling from one segment to another within the internodal myelin sheaths.

2.2.3 Remyelination after demyelination

In some cases, demyelination can occur in the vertebrate nervous system, due to either natural or pathological reasons (e.g. MS). However, after injury or degeneration of white matter, adult neural stem cells are recruited, and will further migrate and differentiate into mature OLs. The "newborn" OLs help to rebuild the myelin structures that support rapid signal transduction through axons. As opposed to myelination, which starts during late embryonic and early postnatal stages, this progress is termed "remyelination". Most genes that regulate either myelination or remyelination are the same (Franklin, 2008), with a few key genes such as *olig2* determining the fate of the OPCs.

Formation of myelin

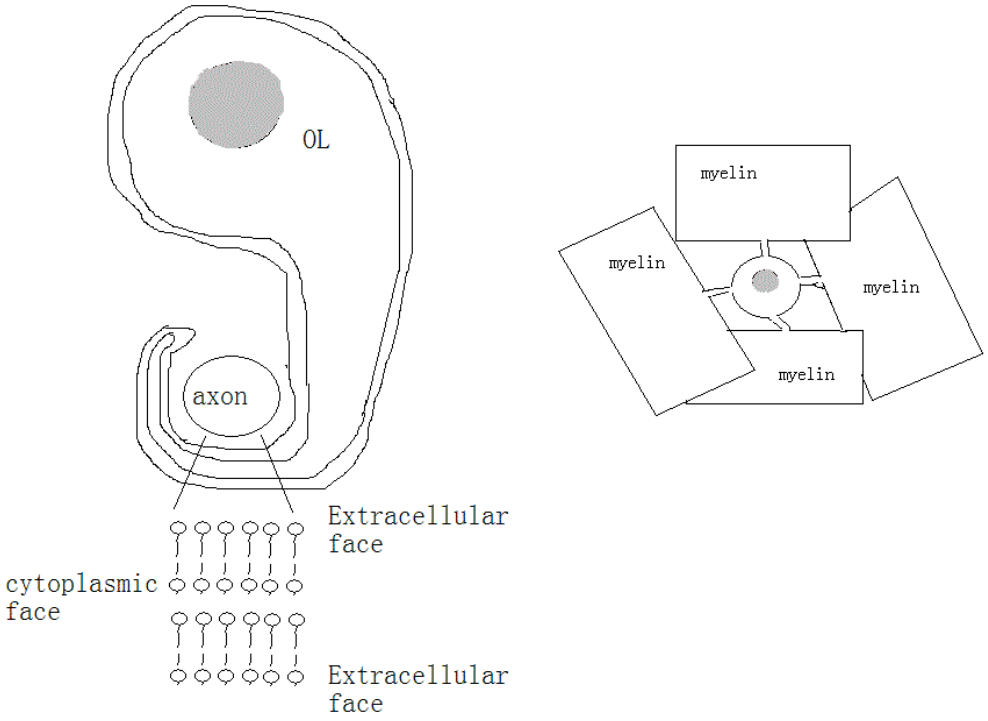


Figure 6. Formation of myelin. Myelin is a fatty layer around the axon. (A) The processes of an OL wrapping then insulating an axon. (B) Each OL can myelinate several axons. Myelin sheaths are part of the processes of OLs (adapted from Verkharatsky and Butt, 2007).

Once demyelination occurs, the system undergoes spontaneous recovery. However, knowledge on this spontaneous remyelination is very limited as it even occurs under normal conditions. Recent research indicates neural genesis can take place following the death of functional cells in adult brain (Bartzokis, 2004; Bartzokis, *et al.*, 2004). The main sources for adult neural genesis are the hippocampus, SVZ, and olfactory bulb (OB) (McMahon and McDermott, 2001; Nait-Oumesmar, *et al.*, 2008). In addition, remyelination mechanisms may be similar to myelination during development, which itself is not entirely understood. However, remyelination fails in demyelination diseases such as MS, suggesting unknown factors can modify the process.

Research demonstrates over-expression of TFs such as Olig1, Olig2, Sox10, and Notch signaling can promote OL specification and differentiation (reviewed in Nicolay, *et al.,* 2007), suggesting the decline of these factors may reduce maturation of OLs in adult CNS. Indeed, researchers using animal models have illustrated the stepwise generation of adult OPCs, which strongly demonstrates the possibility for remyelination in the adult CNS (Bartzokis, 2004; Bartzokis, *et al.*, 2004).

2.3 Diseases related to demyelination

Due to the consequent abnormal behaviors and cognitive damage, the pathology of demyelination diseases in humans has become one of the most popular areas of scientific investigation. Diseases such as MS, stroke, Alzheimer's disease (AD) and schizophrenia are strongly related to demyelinated lesions. Various animal models have been developed to study these diseases (see below).

2.3.1 Multiple Sclerosis

The corpus callosum (CC) is the major white matter tract that crosses the homologous hemispheres in the vertebrate brain (Connor, 2004; Noble, 2004). It consists of approximately 200 million interhemispheric fibers, most of which connect homologous regions of the cerebral hemispheres (Biegon and Eberling, 1994). The CC acts as an

essential bridge that provides the connection between these regions, and integrates sensory, motor, and cognitive information (Steinman, 2001). Since an impaired CC may have less fiber and reduced functional connectivity between the hemispheres, differences in the size of the corpus callosum may lead to distinct cognitive consequences (Gootjes, *et al.,* 2006).

MS is a chronic inflammatory demyelination disease in the CNS. Recognized more than 100 years ago, it is the most widely investigated demyelination disease in the world. Massive demyelination has been identified in the MS brain, followed by extreme macrophage activation. However, the pathology of MS has not been clarified. One clue is the massive demyelinated lesions found in CNS white matter, particularly in the CC (Steinman, 2001). Many animal models have been created to study demyelination and remyelination. In these models, viruses, toxins, or myelin protein antigens are introduced to mimic demyelination and inflammation (Arnett, *et al.*, 2004; Doucette, *et al.*, 2010; Franklin, *et al.*, 2002). Further investigations have also revealed differences of OL lineage cells in treated animals compared to controls (Rist and Franklin, 2008; Sim, et al., 2002).

As mentioned above, OLs must play an important role in the pathogenesis of MS due to the correlation between MS and white matter dysfunction. Recent research indicates OPCs are typically present in demyelination lesions but do not undergo normal maturation, suggesting the inability of OPCs to differentiate (Raine, 1997). In addition, human endogenous retrovirus glycoprotein-mediated induction of redox reactants can cause OL death, which may be another factor in MS pathology (Antony, *et al.,* 2004). Moreover, the apoptotic death of OLs is now being considered as a key step in the initiation of MS development, which may progress to autoimmune attack (Artemiadis and Anagnostouli, 2010). A strong relationship between OLs and MS exists although the actual mechanisms that determine the initiation and progression of MS are not clear.

2.3.2 Stroke

Stroke is a rapidly developing disease in the CNS due to the disturbance of blood supply. Both ischemia and hemorrhage can induce stroke in human brain. Consequently, the affected regions lose normal function (Chiti, *et al.*, 2009). With high metabolic activities, OLs are particularly vulnerable to ischemia as decline of mitochondrial function induces OLs to undergo apoptosis. In addition, death of OLs can lead to axonal degeneration (Chen, *et al.*, 2008); subsequent disruptions of the nerve fibers may cause severe abnormal behaviors (Chen, *et al.*, 2008). A recent study found OL dysfunction in anterior optic nerve ischemia, suggesting the important role of OLs in maintaining the CNS environment as well as stroke prevention (Dratviman-Storobinsky, *et al.*, 2008; Goldenberg-Cohen, *et al.*, 2005).

2.3.3 Neurological disorders

Demyelination is also implicated in AD and schizophrenia (see below). In human brain, myelination continues for the first fifty or sixty years of life (Berlet, *et al.*, 1980). However, OLs are very vulnerable due to their high metabolic activity (Arnett, *et al*., 2004). Demyelination in the white matter is correlated with disruption of neural impulse transmission, which impairs high cognitive functions such as memory (Bartzokis, 2004). Thus, death or dysfunction of OLs is considered to be involved in the pathological processes of neurological disorders such as AD and schizophrenia (Bartzokis, *et al.*, 2001; Seqal, *et al.*, 2007).

2.3.3.1 Alzheimer's disease

AD is a distinctive human disease that typically presents in older adults. In humans, the pathologic lesions of AD may appear as early as the fourth decade of life, during which neuronal loss is not typically observed (Breteler, *et al.*, 1992). To mimic AD, varieties of non-human models have been established, in both genetic and non-genetic forms (Bartzokis, 2004). However, an animal model that includes all AD features has not yet been developed. Recent research indicates multiple genetic and environmental risk

factors may contribute to AD pathology (Lopez, 2001; Schultz, *et al.*, 2000). In particular, detection of increased amyloid β-peptide (Aβ) deposition provides early evidence of AD pathogenesis (Selkoe, 1999; Thal, *et al*., 2002). However, other studies indicate AD symptoms may not appear until later years in the life even with accelerating Aβ accumulation (Vlkolinsky, *et al.*, 2001).

Brain size does not independently contribute to AD, as other animals with large brains, such as elephants and dolphins, do not develop AD during their life spans. In addition, the size of particular brain regions, such as the temporal and frontal lobes, is not a risk factor as other higher primates do not develop AD (Semendeferi, *et al.,* 2002). Generally, age seems to be the only risk factor for developing AD; however, white matter has also recently been identified as a risk factor due to its greater volume in human brains in comparison to other animals including higher primates (Semendeferi, *et al.*, 2002). OLs in the CNS follow a normal age-related schedule to myelinate axons until the fifth decade of life (Bartzokis, *et al.*, 2001). The late-myelinated regions are especially susceptible to development of AD, due to the ease of formation of amyloid-rich extracellular neuritic plaques (NP) and tau-rich intraneuronal neuronfibrillary tangles (NFT), which accelerate AD development in these regions (Thal, *et al.*, 2002; Braak, *et al.*, 2000).

Recent considerable evidence suggests myelin degeneration contributes to AD development. First, after myelin degeneration the transport of cholesterol decreases, which results in accumulations in particular regions (O'Brien and Sampson, 1965). The abundant cholesterol may promote the aggregation of hydrophobic ends of Aβ due to its low capacity to bind water. Second, OLs have the highest iron content in the CNS. The increased iron levels are required for OLs to myelinate more axons in the aged CNS. Iron is also critical to maintain normal protein function and enzyme activity. Recent studies indicate increased iron promotes the oligomerization of Aβ, which further facilitates AD development (Morath, *et al.,* 2001; Power, *et al.*, 2002). In addition, research

demonstrates Aβ preferentially accumulates in unmyelinated or late-myelinating regions, which are involved in short-term memory formation (Tallon-Baudry, *et al.*, 2001). These studies reveal a strong relation between myelin and AD. Thus, dysfunctional OLs and abnormal myelin are implicated in the development of AD but the precise mechanism has not yet been resolved.

2.3.3.2 Schizophrenia

Schizophrenia is a serious mental disorder that can affect the normal life of a patient. Schizophrenia patients can suffer from hallucinations, delusions, and disordered thinking as positive symptoms, as well as avolition, anhedonia and apathy as negative symptoms (Seqal, *et al.*, 2007). In addition, the patient's memory, motivation, and language skills can also be disturbed (Seqal, *et al.*, 2007).

A recent study reveals abnormal white matter in the brain plays a key role in schizophrenia. The dysfunction of white matter is proposed to result in cognitive deficits and impaired memory (Biegon and Eberling 1994). Research indicates white matter is somehow disorganized in schizophrenia. Interestingly, the *Sox10* gene is highly methylated in the brains of schizophrenia patients (Maeno, *et al*., 2007; Iwamoto, *et al.*, 2006). Thus, epigenetic modification of OLs may result in white matter dysfunction. As Sox10 promotes OL maturation, the decreased expression of Sox10 may lead to repression of other OL genes. Indeed, myelin-related genes such as *MAG* and *CNP* are detected at decreased expression levels in schizophrenia (Voineskos, *et al.*, 2008; Mitkus, *et al.*, 2007). Moreover, electron microscopy studies further confirm the OL pathology hypothesis of this disorder. For example, apoptotic OLs and abnormal myelin sheaths have been detected in schizophrenia brains (Bernstein, *et al*., 2009). In addition, the absolute number of OLs is significantly decreased, which also supports the OL pathology hypothesis for schizophrenia (Bernstein, *et al*., 2009; Butts, *et al.*, 2008).

2.3.4 Animal models for the study of demyelination and remyelination

An important approach to study the mechanisms underlying remyelination after degeneration of myelin sheaths is the use of animal models. Several animal models have been developed to study the morphological, cellular, and molecular mechanisms that regulate remyelination after demyelination in young animal CNS. Each model is designed to mimic the pathologies of demyelination disorders such as MS, the most common demyelination disease in humans. However, none of these animal models exactly define all characteristics of MS.

At present, the most common animal models include: 1) toxin-induced demyelination models, such as the cuprizone model that provides a systemic and chronic demyelination (Arnett, *et al.*, 2004; Matsushima, *et al.*, 2001; Doucette, *et al*., 2010), and lysolecithin or ethidium bromide (EtBr) (Skihar, *et al.*, 2009; Yajima and Suzuki, 1979) that produce rapid demyelination; 2) autoimmune-induced demyelination models, such as EAE (Traugott, *et al.*, 1985; Yang, *et al*., 2010); and 3) viral-induced demyelination models (Carlson, *et al.*, 2010; Lang, *et al*.,1984).

Virus models are also widely used to investigate demyelination and remyelination because viruses can induce inflammatory demyelination disease in the CNS. To study demyelination, viruses are injected to induce infection. The most commonly used viruses are Theiler's murine encephalomyelitis virus (TMEV), Semliki forest virus (SFV), and mouse hepatitis virus (MHV). Briefly, TMEV is natural pathogen of mice. Demyelination is observed in the TMEV model with the destruction of OLs (Carlson, *et al.*, 2010; Rodriguez, *et al.*, 1983). In the MHV model, auto-reactive T cells are generated due to inflammation (Baumann and Pham-Dinh, 2001). Compared to other virus-mediated models, SFV is easier to administer due to its ability to penetrate the blood-brain-barrier (Mokhtarian, *et al*., 1999).

The EAE model is widely used in the study of MS, as it produces both inflammation and demyelination. It can be induced by either myelin protein-mediated immune reaction

or T cell-mediated inflammation in the CNS (Bradl and Linington, 1996). However, the period between sensitization and onset of MS depends upon both genetic and non-genetic factors. Recently, more and more myelin proteins have been identified and used as antigens to induce MS pathology in the EAE model, these include MBP, PLP, MAG, and MOG (Bradl and Linington, 1996). However, MOG is the only one at present that can induce both the T cell-mediated response and the myelin protein mediated reaction in EAE model (Baumann and Pham-Dinh, 2001). Interestingly, MOG is critical for the induction of demyelination as only inflammation has been found in MBP-induced EAE (Baumann and Pham-Dinh, 2001). However, massive demyelination is present after intravenous injection of the anti-MOG antibody (Linington, *et al.*, 1988).

Compared to inflammation models, toxin-induced animal models provide the opportunity to independently test demyelination. In particular, EtBr and lysolecithin are commonly used to produce rapid onset demyelination (Franklin, *et al*., 2002; Franklin, 2008; Hinks and Franklin, 2000). Research based on using chemical injection verifies the rapid recruitment of OPCs as well as the steps in OL maturation (Franklin, *et al*., 2002; Franklin, 2008; Hinks and Franklin, 2000).

Another toxin-induced model, the cuprizone model, has been widely used to investigate remyelination responding to chronic demyelination. In this model, young adult mice fed with cuprizone, a copper chelator (Matsushima and Morell, 2001; Doucette *et al.*, 2010), show massive degeneration of myelin sheaths in the CNS white matter, particularly in rostral corpus callosum (RCC) and superior cerebellar peduncle (SCP) (Komoly, *et al.*, 1984; Stidworthy, *et al.*, 2003; Doucette *et al.*, 2010). In addition, spontaneous remyelination is observed as early as 4 days after cuprizone withdrawal, verifying the important role cuprizone plays in this model. Indeed, to sustain the high expenditures due to their large volume of cytoplasm, OLs require more energy compared to other cell types. Thus, OLs are extremely susceptible to cuprizone, as copper is critical for proper mitochondrial function (Torkildsen, *et al*., 2008). Moreover, the C57BL/6

mice strain is commonly used due to its genetic and phenotypic uniformity (Torkildsen, *et al.*, 2008). Recent research suggests the dose of cuprizone in regular chow should be around 0.2% to 0.3% by weight, as only OLs but not other cell types such as astrocytes in the CNS are affected at this dosage (Torkildsen, *et al.*, 2008). Additionally, both MS and EAE involve complex immune response and T cell activation. However, T cells are almost absent in cuprizone-induced demyelination model. Recent studies indicate that for some MS patients, death of OLs is the earliest change in some lesions, which challenge the previous autoimmune theory. Thus, cuprizone model provide supplemental information to studies of demyelination and remyelination processes.

The animal models discussed above make possible the investigation of the stepwise development of OPCs as well as the mechanisms of demyelination and remyelination. However, novel technologies and animal models need to be developed to further elucidate the mechanism of age-related remyelination. Gene expression profile assays and a further understanding of protein structures will contribute to better definition of these processes.

2.4 Aging and CNS remyelination

Recent research demonstrates OPC recruitment occurs rapidly in response to demyelination lesions in the young adult CNS, followed by morphological changes of OPCs into OLs. Ultimately, mature OLs wrap the axons and form novel myelin sheaths. However, efficient recovery is difficult to sustain in aging animals (Shen, *et al.,* 2008).

In general, the ability of OLs to myelinate axons is essential to the proper repair after injury. However, similar to other processes, remyelination efficiency of OLs declines with age (Shen, et al., 2008; Ando, *et al.* 2003; Berlet, *et al.*, 1980). Abundant research over the several years has attempted to explain this phenomenon. Both the decreased rate of OPC recruitment and problems during the differentiation of OPCs have contributed to evolving theories, but alterations of TF expression are likely the reason for

impaired OPC recruitment and differentiation (Franklin, *et al*., 2002; Sim, *et al*., 2002). In addition, the differences in OL lineage cells in old vs. young animals are associated with the age-related expression levels of growth factors, which are essential for proliferation, migration, and differentiation of OPCs (Franklin, *et al.,* 2002). Furthermore, a higher metabolic rate is required for OLs to myelinate an increasing number of axons as well as demyelinating regions. Thus, repair of injured myelin sheaths is especially difficult. Moreover, epigenetic control seems to be involved in many aging related processes (Shen, *et al.*, 2008).

Transgenic techniques are widely used in experiments attempting to explain possible reasons for the age-related decline in myelination efficiency. By over-expressing, truncating, or deleting genes required for OPC recruitment and OL maturation in adult animals, more details are now beginning to be understood (Franklin, *et al.*, 2002; Hinks, *et al*., 2000). Many studies indicate a temporal delay in growth factors in old animals compared to young. These delayed growth factors, such as PDGF and FGF, are critical to oligodendrogenesis and therefore may contribute to the recruitment and maturation of OLs (Franklin, *et al.*, 2002; Hinks, *et al*., 2000).

For many years, it could not be determined whether OPC recruitment or OL maturation determined the slow remyelination rate in aged animals. However, research now shows both are essential. On one hand, there is an age-related decline in OPC recruitment. Studies indicate different numbers of PDGFαR+ cells in the caudal cerebellar peduncles of young and old animals, suggesting the recruitment of OPCs may decrease remyelination (Zhao, *et al.*, 2005). In addition, the rate of OPC recruitment decreases with age, as PDGFαR mRNA levels are significantly lower in older animals (Franklin, *et al.,* 2002). On the other hand, an age-related impairment in OL differentiation has been described, indicating the remyelination process may be halted at this step (Blakemore and Keirstead, 1999). Research indicates a delay in MBP and PLP mRNA expression in old mice after EtBr injection (Franklin, *et al*., 2002), suggesting OL

differentiation is blocked. Moreover, an age-dependent epigenetic inhibition has been described. Research indicates that histone deacetylases (HDACs) are not present in sufficient quantities in aging animals, and thus transcriptional inhibitors accumulate and prevent the subsequent myelin gene expression (Shen, *et al.*, 2008). Macrophages may also contribute to effects of aging on remyelination. A notable difference in cellular environments has been reported (Zhao, *et al.*, 2008). Macrophages can remove myelin debris, which is harmful to remyelination after demyelination (Sun, *et al.*, 2010; Henderson, *et al.*, 2009). Thus a delayed reaction by macrophages in some cases can delay remyelination. Nevertheless, the precise mechanisms still need to be resolved and are dependent on the complicated signaling pathways in the CNS.

III Materials and methods

The following methods and protocols were used to accomplish the objectives identified in Section 1.2.

3.1 Cuprizone

The neurotoxicant cuprizone is a copper chelator that reproducibly induces massive demyelination in large areas of the mouse brain (McMahon, *et al.,* 2001). OLs are especially vulnerable to this neurotoxicant because of their high metabolic activity, which is dependent on copper for proper mitochondrial function. The administration of cuprizone causes cell death of OLs, which leads to extensive demyelination of select myelinated fiber tracts in adult mice (Armstrong, *et al*. 2002). The two best studied fiber tracts are the corpus callosum (Morell, *et al*., 1998; McMahon, *et al.*, 2002) and the superior cerebellum peduncle (Komoly, *et al*., 1992). Review by Torkildsen (Torkildsen *et al.*, 2008) discussed the cuprizone model in detail. In the standard model, young adult mice are fed with 0.2%-0.3% cuprizone in the chow. Under this dosage, OLs seem to be the only glial cell type o be affected in the CNS.

Although cuprizone is a copper chelator; the administration of copper is not an antidote (Carlton *et al.*, 1967). The assumed mechanism of cuprizone to kill OLs is that the cuprizone leads to inhibition of the copper-dependent mitochondrial enzymes cytochrome oxidase and monoamine oxidase (Matsushima and Morell, 2001).

3.2 Animal Protocols

Cuprizone is ideal for studying remyelination because cuprizone-induced demyelination is easily inducible, localized, and predictable. In addition, cuprizone model only associates with microglia/macrophages response but not T cell activation, which mimic early MS symptoms (Baumann and Pham-Dinh, 2001). In this project, we developed the classic model using cuprizone to induce demyelination in mice. Instead of using only young animals, the extension of age was designed as a factor to alert the response of OLs to demyelination lesions.

With the experimental design (Figure 7), there were three groups: a) control mice, b) cuprizone-treated mice, and c) cuprizone recovery mice. Young adult (2-month old), adult (6-month old), and aged (12, 16-month old) C57BL/6 mice were fed with cuprizone-treated (0.2% by weight, mixed with their diet of milled chow) or control food for a period of 6 weeks. At the end of the 6th week, half of the animals (n=9) were sacrificed, and the remainder (n=9) fed normal food for an additional 3 weeks (for myelin recovery). Animals were euthanized by perfusion (for immunohistochemistry, n=3) or dissected brains were fresh frozen (for Quantitative Real Time PCR, n=3) (Figure 7).

3.3 Perfusion

Three animals from each group were perfused for further immunohistochemical study. The mice were perfused through the heart under deep anesthesia. The mice were first perfused with phosphate buffered saline (PBS), and then pefused with cold freshly made 4% paraformaldehyde (pH 7.4). After perfusion, brain tissues were quickly removed and post-fixed in 4% paraformaldehyde overnight (Doucette et al., 2010). The next day the tissues were transferred into 30% sucrose for storage.

3.4 Immunohistochemistry

Whole half brains were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek), and serially sectioned (8 μm) in either coronal or sagittal planes with a cryostat onto gelatin coated coverslips (Figure 8). The sections were air dried at room temperature for 30 min，and kept at -20°C for further use. For immunohistochemical study, the sections were rehydrated in PBS for 2 x 10 min. The sections were then blocked in 3% SM-PBST (3% skim milk and 1% Triton X-100 in PBS) for 40 min. Sections were then exposed to the diluted primary antibody in PBS at 4°C overnight. Primary antibodies were diluted in PBS. The sections from each animal were exposed to MOG and MBP as single staining, PDGFαR/Olig2, PDGFαR/GFAP, Olig2/GFAP as double labeling. Table 1 lists the titers of the antibodies employed. The unhybridized primary antibody was then rinsed off twice with PBS. The secondary antibody was then

applied for 45 min. at room temperature. Distinctive secondary antibodies were employed to hybridize primary antibodies (see Table 1). After two washes in PBS, the sections were counterstained with Hoechst dye (Sigma) to visualize the nuclei. Finally, the sections were rinsed twice in PBS, mounted in Prolong (Molecular Probes), and visualized with fluorescence microscopy.

3.5 Microscopy

At least 20 consecutive tissue sections were collected at intervals of 300 μm, where CC begins to cross rostrally at the anterior commissure. Olympus CKX41 light microscopy was used to image the immunohistochemical stained tissue sections with objective lens magnification of either X10 or X20. Images were taken with a Nikon CoolPix4500 digital camera. Fluorescent images were observed with an Olympus BX40 filter system with appropriate filter sets. Under fluorescence microscopy, the rostral corpus callosum (RCC) of each section was captured by digital camera. For MOG staining, images were taken with X10 objective lens. For double staining, three adjacent images within the medial aspects of the RCC (500 μm^2) were captured from the midline of the brain, each section were imaged using X20 objective lens. All images were saved on the computer hard drive and further analyzed with Image-Pro Express® software (Media Cybernetics).

3.6 Blinded Cell Counts

After immunofluorescent staining, each section was assigned a code and all cells were counted blindly. Sections of PDGFαR/Olig2 and GFAP/Olig2 double labeling were analyzed by testing cell population. Cells were counted manually using Image-Pro Express® software. The numbers of double stained cells in each image were counted in terms of as nuclear expression (eg. $Olig2^{Nuc}$+ve), cytoplasmic expression (eg. $Olig2^{Cyto}$+ve), and any co-expression. The data collected from the sections were total cell density, co-expression of $Olig2^{Nuc}$+ve/PDGFαR+ve, $Olig2^{Nuc}$+ve/GFAP-ve,

PDGFαR+ve/GFAP-ve, Olig2^{Cyto}+ve/GFAP+ve, Olig2^{Cyto}+ve/ PDGFαR+ve, and Olig2^{Cyto}-ve/GFAP+ve. Total cell density was visualized by Hoechst stained nuclei.

3.7 mRNA Isolation and Reverse Transcription (RT)

Specific areas of each mouse brain, including the anterior corpus callosum, posterior corpus callosum, anterior hippocampus, posterior hippocampus, and cortex were selected from frozen fresh brains. However, only the anterior CC was used for further studies. Total mRNA was isolated using a RNeasy Protect Mini Kit (Qiagen) according to the manufacturer's protocol. RNA concentration was determined by UV spectrophotometry (Ultrospec 3100 pro). First strand DNA synthesis (RT) was performed with the SuperScript® first strand synthesis kit (Invitrogen) with random primers using the manufacturer's protocol. The final concentration of total RNA for RT was 20 ng/μL.

3.8 Quantitative RT PCR

Specific factors such as MOG, Olig2, CGT, Nkx2.2, PDGFαR, and β-actin (as internal standard) were detected by quantitative RT-PCR (7300 Biosystems) using commercially available primers (Applied Biosystems). All reactions are performed using the Taqman Universal Master Mix, FAM-labeled Taqman Gene Expression assays for genes of interest, VIC-labeled Taqman Endogenous Control β-actins, and 10 ng of cDNA as a template. All reactions were run in replicates of 4, and n=3 animals were used for each gene per group. Thermocycling parameters were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 70 s at 60°C. All comparisons are conducted with relative quantification software (Applied Biosystems). Related Quantitative (RQ) values were obtained by quantitative RT PCR, and normalized to the mean value of the respective 2 month control mice. Normalized RQ values greater than 1 indicate increased gene expression, while RQ values less than 1 indicate decreased gene expression levels compared to the 2 month control.

3.9 Statistical analysis

For immunohistochemistry experiments, cell density was calculated for the data from each treatment group. Data was further analyzed by two-way ANOVA followed by Bonferroni's post-hoc multiple test (GraphPad Prism). Data for MOG, CGT, PDGFαR, Olig2, and internal control (β-actin) from quantitative real time PCR were analyzed by two-way ANOVA followed by Bonferroni's post-hoc multiple test (GraphPad Prism). A *p* value less than 0.05 was considered to be significant.

Schematic diagram illustrating experimental design of this study

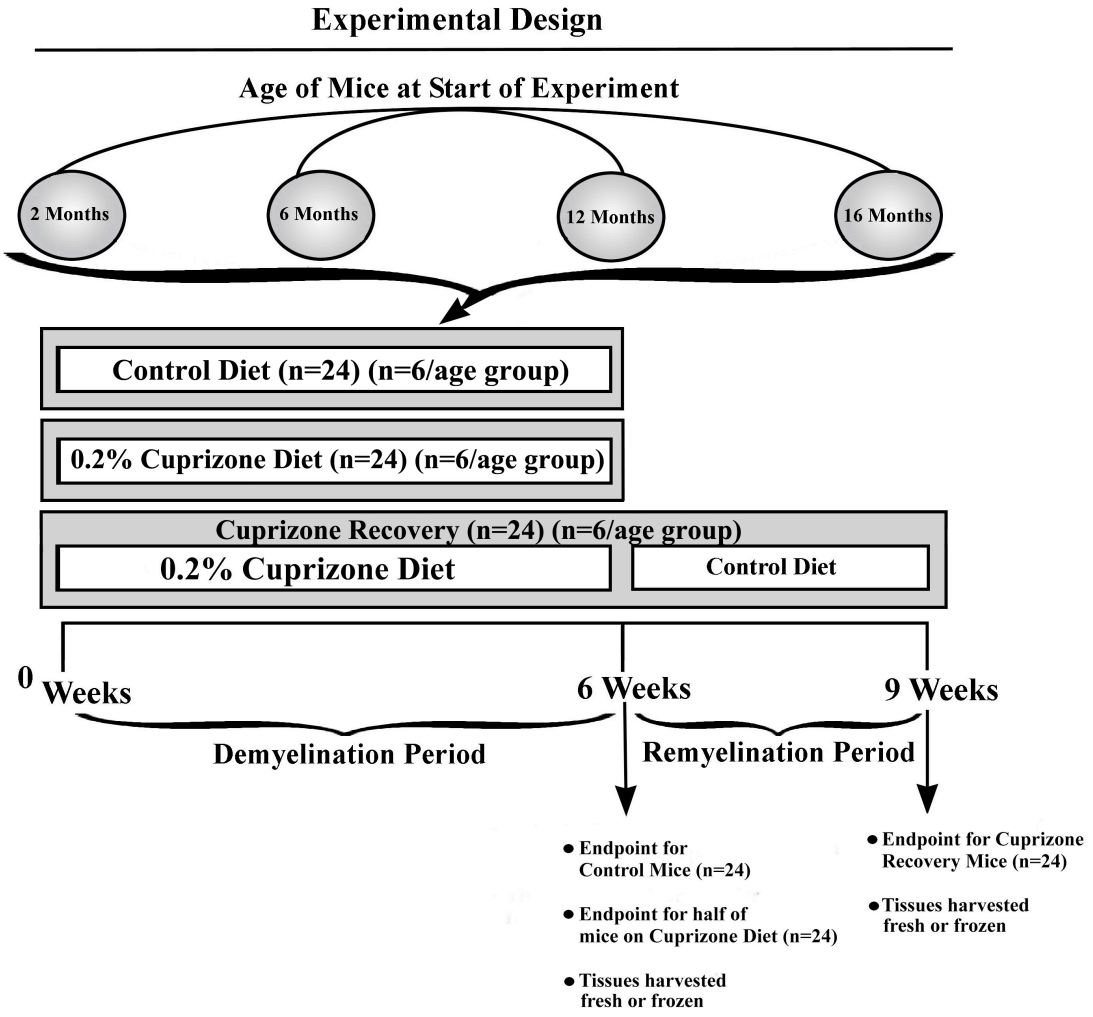


Figure 7. A diagram illustrating experimental design of the study. There were three treatment groups: a) control mice, which were fed normal milled chow for 6 weeks; b) cuprizone mice, which were fed a 0.2% cuprizone-containing diet for 6 weeks; and c) cuprizone recovery mice, which were fed 0.2% cuprizone diet for the first 6 weeks followed by normal milled chow for the next three weeks. Six mice were used for each of the three treatments at each of the four ages. Half of the mice for each treatment and age were used for immunofluorescent microscopy and the remaining half for collecting tissue for quantitative RT-PCR (qRT-PCR).

Table 1. Titers of the antibodies used in immunohistochemistry

Primary antibody		Secondary antibody	
Antibody	Titer	Antibody	Titer
PDGF α R (BD Pharmingen)	1:100	Alexa Fluor 594 goat anti rat IgG	1:400
GFAP (Dimensionlabs)	1:1000	Alexa Fluor 488 goat anti rabbit IgG	1:200
GFAP (Sigma)	1:1000	Alexa Fluor 488 goat anti mice IgG	1:200
Olig2 (Takebayashi)	1:1000	Alexa Fluor 594 goat anti rabbit IgG	1:400
MBP (Sternberger)	1:1000	Alexa Fluor 594 goat anti mice IgG	1:400
MOG (Gardinier)	1:3	Alexa Fluor 594 goat anti mice IgG	1:400

Schematic diagram to show tissue section and collection

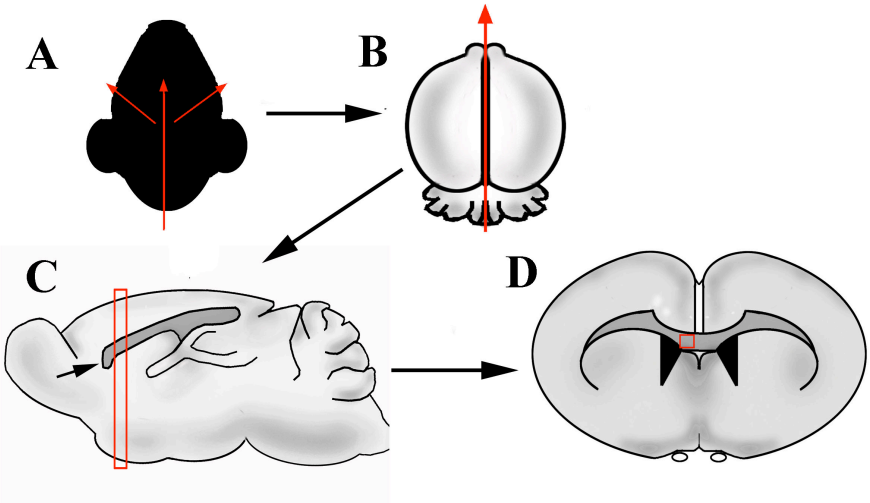


Figure 8. A diagram summarizing how the brain was dissected for cutting sagittal and coronal cryostat sections. 'A' The brain is removed from the skull and separated down the midline ('B'), thus dividing the brain into a right and a left half. 'C' shows a midsagittal section of the right cerebral hemisphere, including the genu (arrow) and body of the corpus callosum. Myelin protein immunostaining was done on cryostat sagittal tissue sections to see age-related differences in cuprizone-induced demyelination in the corpus callosum and the extent of myelin repair in the cuprizone recovery mice. The long, thin rectangle passes through the rostral part of the body of the corpus callosum and indicates the plane of section for the drawing in 'D', which shows the coronal plane through the brain. This coronal plane was used for all of the immunostaining for quantifying cell densities, with the quantification done on digital images captured from the medial part of the corpus callosum, which is indicated by the boxed area in 'D'. Three adjacent images were taken from the midline of the brain, medially to laterally. The corpus callosum tissue used for qRT-PCR was obtained from a comparable area within the medial aspect of this myelinated fiber tract.

IV Results

4.1 Health condition of mice fed the control or cuprizone diet

Each mouse was weighed daily until they were killed for the study. The daily weighing enabled us to trace the effect of the cuprizone diet on their eating habits as well as the normal weight with aging. We also observed the behavior of the mice, and found nothing unusual in both control and cuprizone fed mice. With the exception of body weight loss, and a few dermatitis problems, all the mice were considered healthy until they were killed for immunostaining or quantitative RT PCR.

4.2 Effectiveness of cuprizone-induced demyelination of RCC

To test the effectiveness of cuprizone contained diet on mice aged 2-16 months old, coronal and sagittal sections from control and cuprizone-fed mice were immunostained with MBP and MOG antibodies. Under fluorescent microscopy, both MBP and MOG staining showed a similar expression pattern of demyelination in the RCC of mice of all ages (Figure 9, 10 and 11). MBP and MOG positive expression indicate myelin sheaths, which represent mature OLs.

Mice were fed a cuprizone-containing diet for 6 weeks to develop demyelination. Spontaneous remyelination was expected after the withdrawal of cuprizone diet after 3 weeks. As expected, massive demyelination was observed in the RCC of mice after 6 weeks of cuprizone fed. The immunostaining indicate a similar loss of MBP and MOG in the cuprizone fed mice of all 4 ages. As well, no age-related difference was observed in the control mice across all ages. However, noticeably less immunostaining of MBP and MOG was found in the RCC of 12 and 16 month-old cuprizone recovery mice compared to the 2 and 6 month-old cuprizone recovery mice. The immunostaining of MBP and MOG suggests that there may be age-related differences in OPCs recruitment or OL maturation, as well as altered myelin gene expression (Figure 9, 10, and 11).

Immunohistochemical analysis of MBP in the RCC of 2, 6, 12, and 16 month-old mice

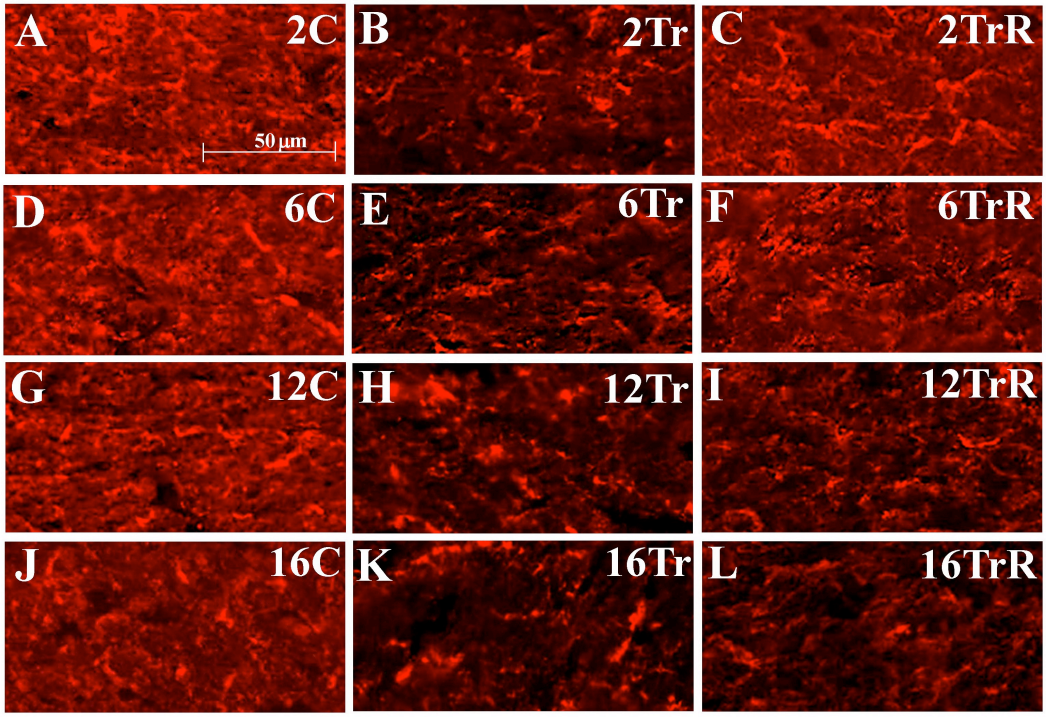
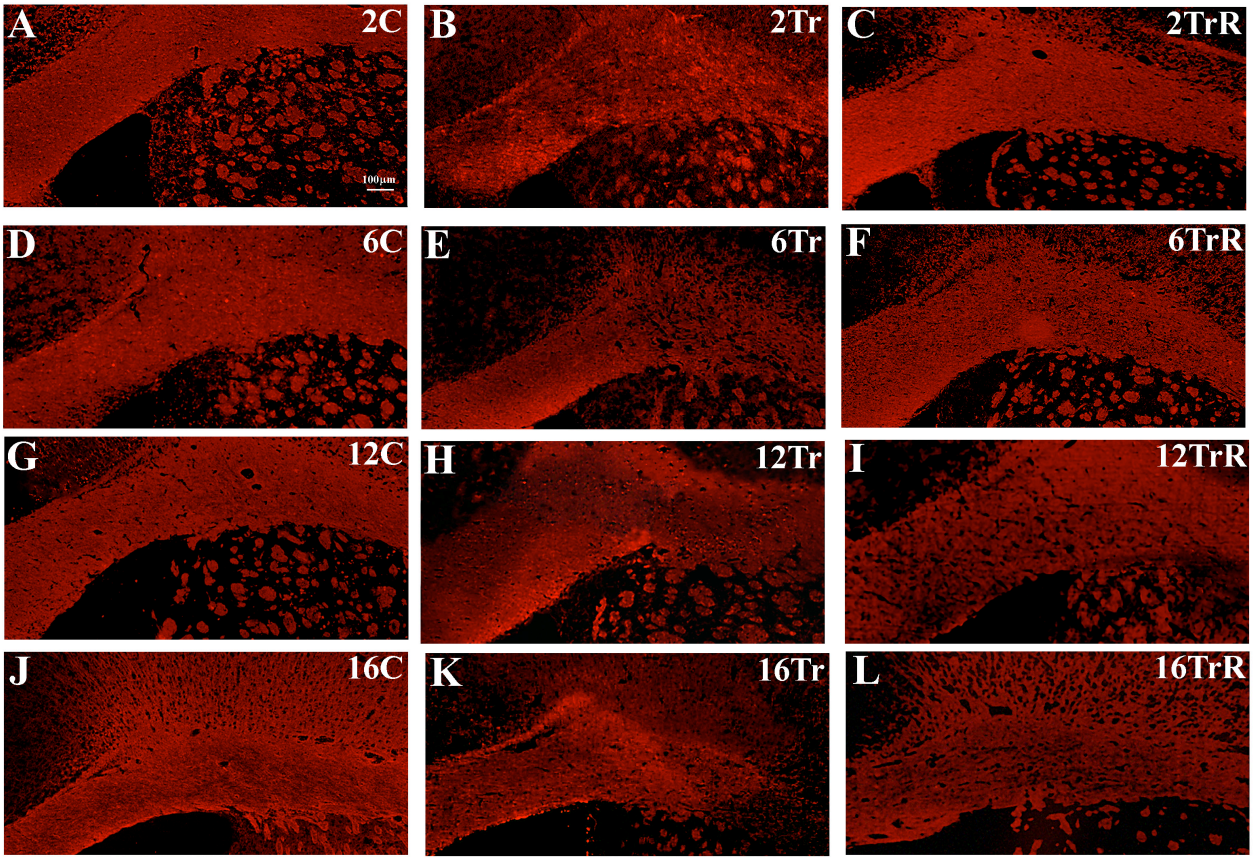


Figure 9. Immunohistochemical analysis of MBP in RCC of 2, 6, 12, and 16 month-old mice: untreated control (A, D, G and J), 6 week cuprizone treated (B, E H and K), and 6 week cuprizone treatment followed by 3 week recovery (C, F, I and L). An age-related decline in MBP expression is evident (A, D, G and J). Cuprizone fed mice show decreased expression of MBP in the RCC (B, E, H, K compared to A, D, G, J) that recovers in 2 and 6 month old mice (C, F compared to A, D) after a period of 3 weeks but does not recover as well in 12 and 16 month old mice (I, L compared to G, J). n=3, bar=50 microns.

Immunohistochemical analysis of MOG in coronal sections through the CC of 2, 6, 12 and 16 month-old mice



bar=100 microns

Figure 10. Immunohistochemical analysis of MOG in coronal (A-L) sections through the CC of 2, 6, 12 and 16 month-old mice: control (A, D, G, and J), cuprizone (B, E, H, and K), cuprizone recovery (C, F, I, and L). Cuprizone fed mice show decreased expression of MOG in the RCC (B, E, H, K compared to A, D, G, J) that recovers in 2 and 6 month old mice (C, F compared to A, D) after a period of 3 weeks but does not recover as well in 12 and 16 month old mice (I, L compared to G, J). n=3, bar in 'A' = 100 microns (applies to 'A' to 'L').

Immunohistochemical analysis of MOG in sagittal sections through the CC of 12 and 16 month-old mice

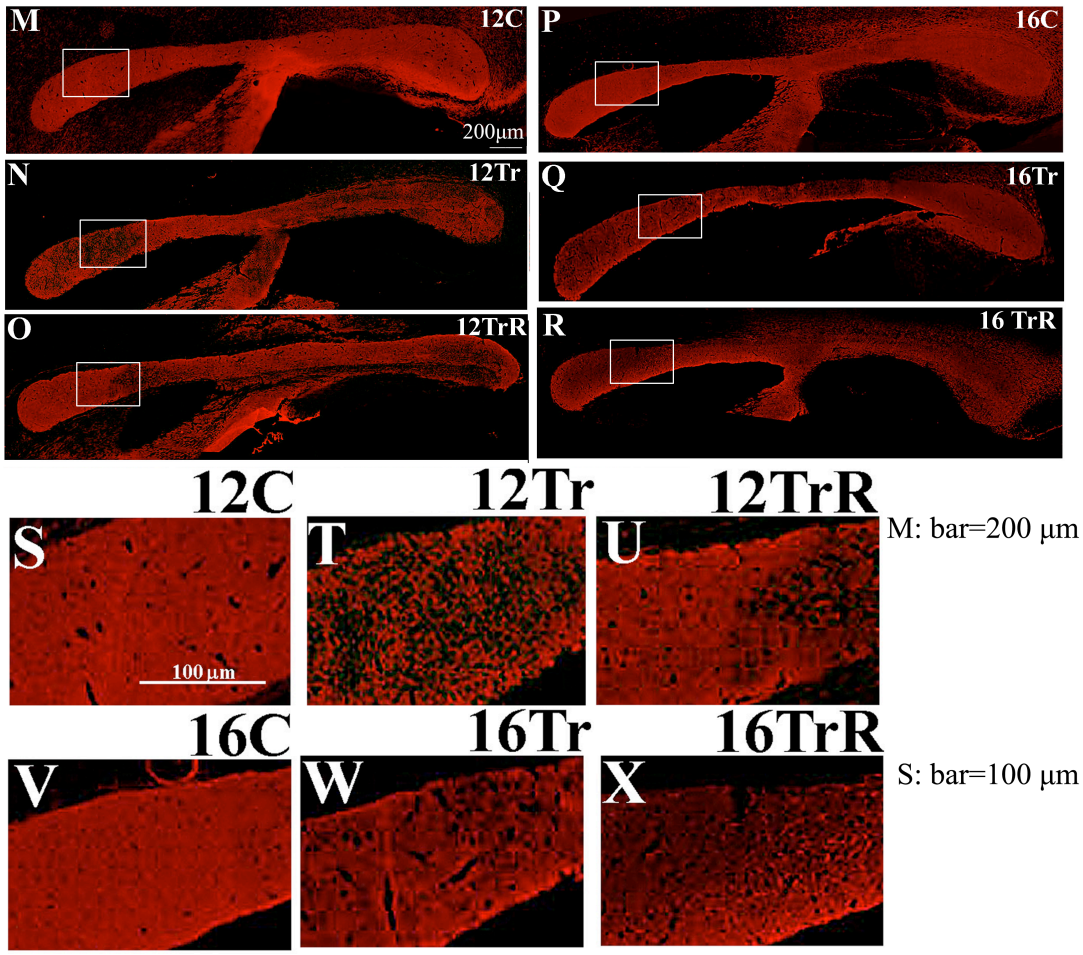


Figure 11. Immunohistochemical analysis of MOG in sagittal (M-X) sections through the CC of 12 and 16 month-old mice: control (M, P, S and V), cuprizone (N, Q, T and W), cuprizone recovery (O, R, U and X) mice. The boxes in 'M' to 'R' indicate the areas in the RCC shown in higher magnification in 'S' to 'X', respectively. Cuprizone fed mice show decreased expression of MOG in the RCC of mice (T,W compared to S, V) that does not recover after 3 weeks in 12 and 16 months old mice (U, X compared to S, V). n=3, bar in 'M' = 200 microns (applies to 'M' to 'R'), bar in 'S' = 100 microns (applies to 'S' to 'X').

4.3 Changes in myelin gene expression during normal aging

To determine if there were age-related changes in myelin gene expression, quantitative RT PCR was employed to quantify the expression levels of myelin genes in the RCC of mice. The expression of two genes quantified were *MOG* and *CGT*. *MOG* expression represents the late stage of OLs that myelinate axons, *CGT* represents the early stage of OL during development, respectively. The RQ values for each gene were normalized to the mean RQ value of the 2 month control mice. The statistical significant differences were assessed using a two-factor ANOVA (Figure 12).

The expression levels of *MOG* in control mice underwent an age-related decrease (Figure 12A). The normalized RQ values for *MOG* at 16 months old were 4-fold lower than 2 months old control mice. As well, there was an age-related decrease of *CGT* gene expression (Figure 12B). However, there was less than a 2-fold decrease of *CGT* expression in contrast to the 4-fold reduction of *MOG* expression in the RCC of these same mice.

4.4 Expression of myelin genes in response to cuprizone-induced demyelination

We predicted that myelin gene expressions in the RCC of mice would be decreased in response to cuprizone treatment. However, the expected reduction of *MOG* gene expression was seen only at 2 month-old group ($p<0.5$) (Figure 12A). In contrast to normal aging, there were no significant differences of *MOG* expression in response to cuprizone-induced demyelination at 6, 12, and 16 months mice. In addition, there were no significant differences of *MOG* expression between 4 ages. Thus, the *MOG* expression in response to cuprizone-induced demyelination was similar at all four ages (Figure 12A).

The RQ values revealed that cuprizone treatment differentially affected *CGT* gene expression in the RCC of mice. The only statistical difference as indicated by Bonferroni's post test was a significant increase of *CGT* expression at 12 month-old, compared to the age-matched control ($p<0.5$)(Figure 12B). However, this function is not

common in older mice since the *CGT* expression level of 16 month-old group was not significant from that of age-matched control mice ($p>0.5$) (Figure 12B).

4.5 Changes in myelin gene expression after three weeks recovery

The gene expression levels of *MOG* and *CGT* after 3 weeks recovery indicate the ability of OLs to repair damaged tissues. Thus any increase of myelin gene expression was an attempt by OLs to recover from cuprizone-induced demyelination in the recovery period. The statistical analysis revealed that the normalized RQ value of *MOG* after recovery was significantly higher at 2, 12, and 16 month-old mice compared to the age-matched cuprizone mice ($p<0.5$) (Figure 12A). The increase of *MOG* gene expression was between 1.8 fold (16 months old) and 2.5 fold (12 months old). In addition, the normalized RQ value of *MOG* was significantly increased compared to age-matched control mice at 12 and 16 month-old mice ($p<0.5$) (Figure 12A). Therefore, there were significant increases of *MOG* expression after 3 weeks of cuprizone withdrawal, even in the older age of the mice.

With respect to *CGT* expression of cuprizone recovery mice, there was no significant difference among most of the RQ values normalized to 2 months control. The only difference was observed at 12 month-old of age. The expression of *CGT* was increased compared to the age-matched control ($p<0.5$) (Figure 12B). In addition, 16 months of age group showed a similar trend but did not reach significance ($p>0.5$). Thus, unlike the *MOG* recovery pattern, aging has less effect on *CGT* expression after 3 weeks of cuprizone recovery mice. The expression of *CGT* gene in response to withdrawal from cuprizone-contain diet was similar at all four ages.

Histograms depicting gene expression profiles for MOG and CGT in the RCC

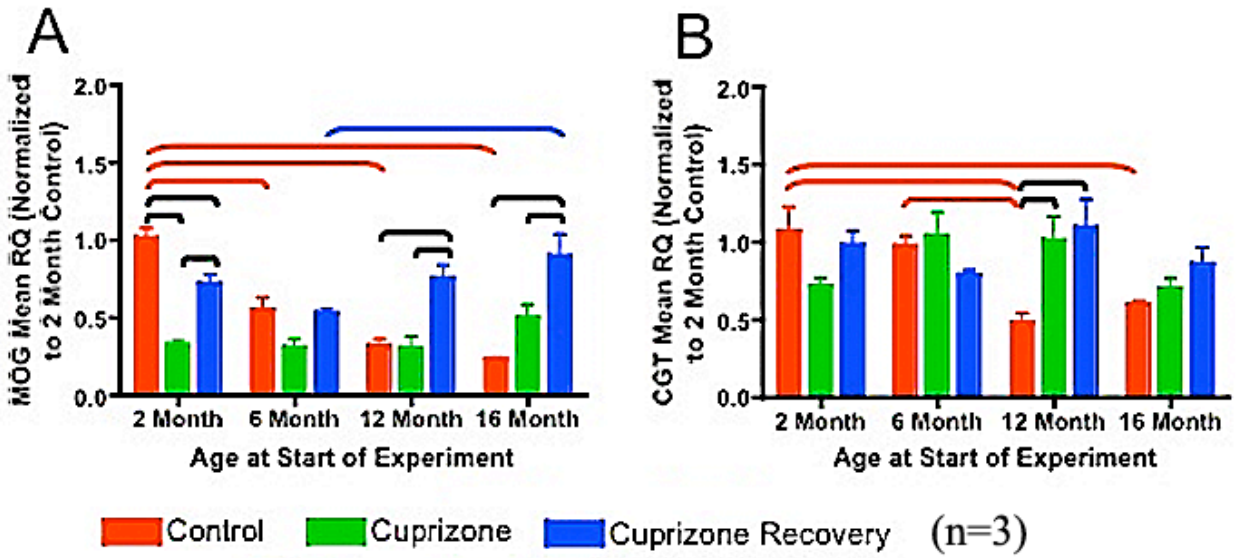


Figure 12. Histograms depicting the gene expression profiles (quantitative [q] RT-PCR) for *MOG* (A), and *CGT* (B) in the RCC of 2-16 month-old control, cuprizone and cuprizone recovery mice (n=3). The qRT-PCR data are expressed as normalized RQ values, with all RQ values for a gene being normalized to the mean RQ value of the respective 2 month-old control mice. For *MOG* expression (A), the two-way ANOVA showed significant main effects of age ($p<0.0001$) and treatment ($p<0.0001$), as well as a significant interaction effect ($p<0.0001$). For *CGT* expression (B), the two-way ANOVA showed a significant main effect of age ($p=0.04$) and a significant interaction between age and treatment ($p<0.0001$); the main effect of treatment was nonsignificant ($p>0.05$). For all histograms, the brackets in color denote significant age-related differences within a group, whereas black brackets denote significant treatment-related differences (Bonferroni's post-test; significance level of $p<0.05$).

4.6 Changes in OL specific gene expression during normal aging

The expression of OL specific genes such as *PDGFαR*, *Olig2*, and *Nkx2.2* are critical to OL development and myelination. In the subsequent experiments, we asked whether there were age-related changes in expression of these genes. The RQ values of *PDGFαR*, *Olig2*, and *Nkx2.2* genes were normalized to that of 2 months old control mice respectively. We employed two-way ANOVA as statistical analysis, in which age and treatment worked as the factors.

We did not find any significant difference in the expression of *PDGFαR* gene in the 2-16 months control mice ($p>0.5$) (Figure 13A). However, a significant age-related reduction of *Olig2* gene expression was observed in the RCC of control mice ($p<0.5$) (Figure 13B). The normalized RQ value at 16 months old was about 3.3 fold lower than that of 2 months control. Compared to the decreased expression of *Olig2* gene, the normalized RQ values of *Nkx2.2* revealed a significant increase in older age of mice. For instance, the RQ value of *Nkx2.2* at 16 months control mice (2.78 +/-0.47) was 2.6-fold higher than that of 2 months old mice (1.06 +/-0.11) ($p<0.5$). Therefore, age affected expression of *Olig2* and *Nkx2.2* differentially.

4.7 Changes in OL specific gene expression during cuprizone treatment

Unexpectedly, *PDGFαR* gene expression was only affected in 2 month-old mice RCC during cuprizone-induced demyelination. There was a significant reduction in RQ value at 2 months cuprizone mice (0.58 +/-0.05) compared to the age-matched control mice (1.0 +/-0.1) ($p<0.5$) (Figure 13A). However, we did find an age-related increase in *PDGFαR* expression in the RCC of cuprizone mice. The expression level in 12 month-old was significantly higher than that in 2 and 6 months old cuprizone mice ($p<0.5$) (Figure 13A).

For *Olig2* gene, the expression was significantly higher in 12 and 16 month-old cuprizone mice compared to the age-matched control ($p<0.5$) (Figure 13B). However, the normalized RQ value of *Olig2* in 2 months cuprizone mice was significantly lower than

the age-matched control. For Bonferroni's post test, the RQ value in 12 month-old mice was significantly higher than that of 2 months cuprizone mice ($p<0.5$) (Figure 13B).

With respect to the expression of *Nkx2.2*, it failed to reach statistical significance between cuprizone mice and age-matched control mice in all four age groups (Figure 13C). Thus we assume that age is the main factor in impacting gene expression of *Nkx2.2* gene. Similar to the control aged mice, the expression of *Nkx2.2* was significantly increased in the older mice compared to the younger aged mice following cuprizone treatment. The RQ values revealed that there were more than 2 fold increases at 12 (2.53+/-0.45) and 16 (2.24 +/-0.44) months cuprizone mice than that of 2 months cuprizone mice (0.95 +/-0.08) ($p<0.5$) (Figure 13C).

4.8 Changes in OL specific gene expression after cuprizone recovery

For *PDGFαR* gene expression, there were no significant changes between cuprizone and cuprizone recovery mice in all four ages ($p>0.5$) (Figure 13A). However, Bonferroni's post test revealed that gene expression level in 12 months old cuprizone recovery mice was significantly higher than that of 2 and 6 month-old cuprizone recovery mice. In addition, the RQ value of *PDGFαR* in 16 months cuprizone recovery mice was significantly higher than that in 6 months cuprizone recovery mice ($p<0.5$) (Figure 13A). Moreover, there was significantly increased *PDGFαR* gene expression in the RCC of 12 months cuprizone recovery compared to the age-matched control mice ($p<0.5$) (Figure 13A).

With respect to *Olig2* gene, expression levels were significantly decreased in the recovery mice at 6 and 16 month-old compared to the age-matched cuprizone mice ($p<0.5$) (Figure 13B). Furthermore, a significant decrease in *Olig2* expression in the RCC of 2 month-old cuprizone recovery mice was revealed compared to the age-matched control mice. However, a significant increase of *Olig2* in 12 month-old recovery mice was observed compared to the age-matched control ($p<0.5$) (Figure 13B).

None of the significant differences in *Nkx2.2* gene expression was detected between cuprizone and recovery mice at all four ages ($p>0.5$) (Figure 13C). However, an age-related increase of *Nkx2.2* gene expression in the RCC of cuprizone recovery mice was observed by Bonferroni's post test. The normalized RQ values of 12 months cuprizone recovery mice were significantly higher than that of 2 and 6 months recovery mice ($p<0.5$) (Figure 13C). In addition, the expression level of *Nkx2.2* at 16 months was up to 5-fold higher compared to 2 and 6 months cuprizone recovery mice ($p<0.5$) (Figure 13C).

4.9 Cell densities of Hoechst +ve nuclei (total cell numbers)

The Hoechst+ve nuclei indentified the total cell number. We counted the cell number in each of three images that were captured from the main area of RCC (Figure 8). Two-factor ANOVA was employed to analyze the aspect of aging and cuprizone treatment. It was shown that there was no age-related difference in total cell density in the RCC of control mice in all four ages ($p>0.5$) (Figure 14A). Due to this result, we confirmed that the main effects of aging on myelin genes were not due to any changes in total cell densities (Figure 14A).

In contrast to control mice, the only statistical difference in total cell densities between cuprizone and control mice was shown at 2 months of age. There was a significant increase in cell number after treatment of cuprizone ($p<0.5$) (Figure 14A). In addition, the total cell densities at 6, 12, and 16 months of cuprizone mice were considerably less than that at 2 months of age ($p<0.5$) (Figure 14A). Therefore, it is possible that the response to cuprizone treatment was impaired in the aged mice compared to the 2 month-old mice.

Histograms depicting gene expression profiles for PDGFαR, Olig2, and Nkx2.2 in the RCC

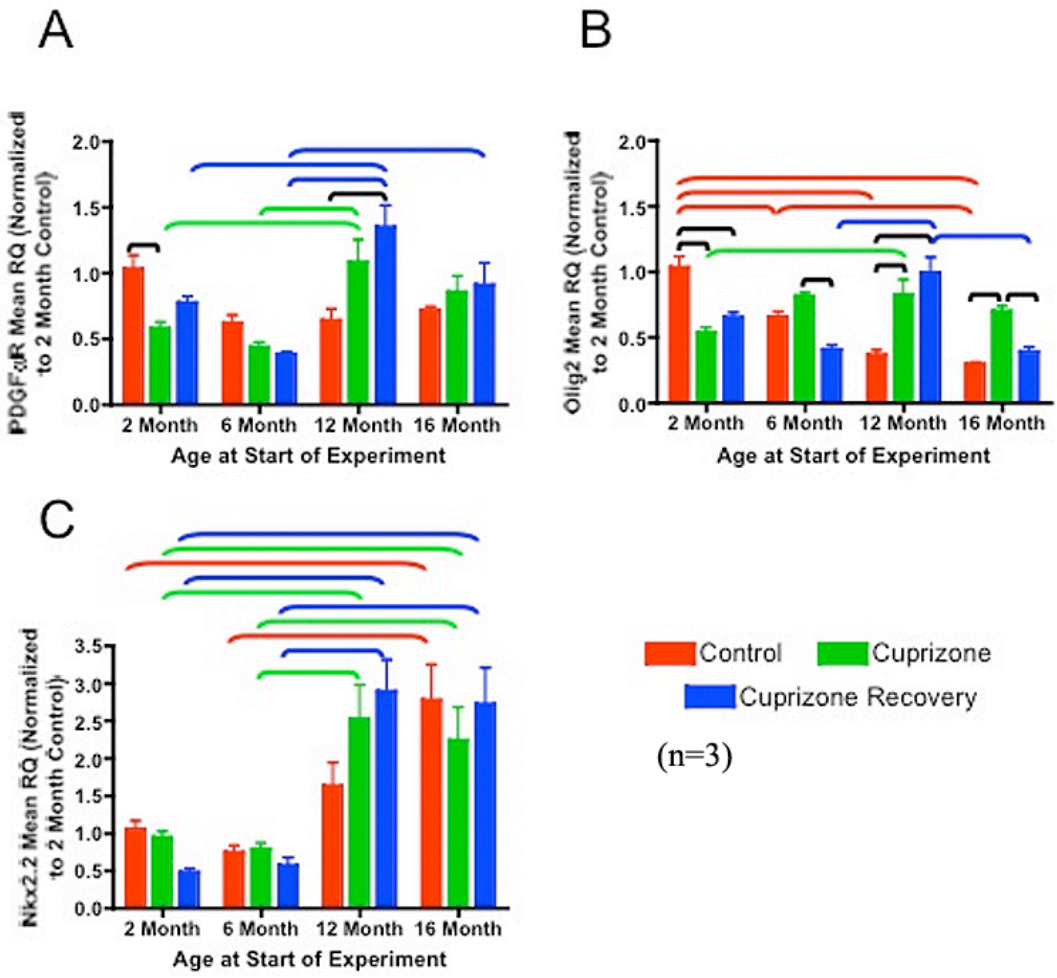


Figure 13. Histograms depicting the gene expression profiles for *PDGFαR* (A), *Olig2* (B), and *Nkx2.2* (C) in the RCC of 2-16 month-old control, cuprizone, and cuprizone recovery mice (n=3). The RQ values were normalized to the mean value of 2 months control mice. A) A significant effect of age ($p=0.0001$) and interaction between age and treatment ($p<0.009$) on *PDGFαR* gene expression was revealed. B) For *Olig2* expression, the two-way ANOVA revealed main effects of age ($p=0.0001$) and treatment ($p<0.0001$), as well as the interaction between age and treatment ($p<0.0001$). C) The two-way ANOVA on the RQ values of *Nkx2.2* showed a significant main effect of age ($p<0.0001$) as well as the interaction between age and treatment ($p<0.05$). The brackets denote significant differences (Bonferroni's post test, $p<0.05$).

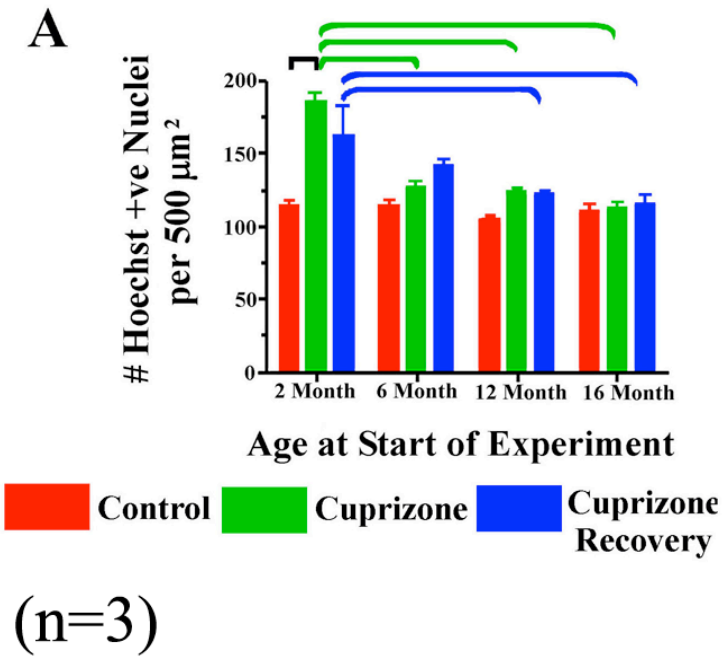
After three weeks of cuprizone withdrawal, the only difference in cell density was noted in 2 months cuprizone recovery compared to the age-matched control. There was approximately 42.6% increase in total cell density ($p<0.5$) (Figure 14A,B,D). In contrast to 2 months of age, the 6, 12, and 16 months cuprizone recovery mice failed to reach the statistical significance in comparison to the age-matched control ($p>0.5$) (Figure 14A,C,E). Interestingly, similar to cuprizone mice, the tissue response of older mice with respect to cell density was considerably lower than that of 2 month-old cuprizone recovery mice (Figure 14A,D,E).

4.10 Changes in density of cells of the OL lineage after cuprizone-induced demyelination and three weeks recovery

In the subsequent experiments, we traced oligodendrogenesis in the RCC. Olig2 functions as a TF in OL lineage cells. As mentioned previously, it plays important role in specification, differentiation and maturation of OLs (Nicolay, *et al*., 2007). Furthermore, the translocation of Olig2 protein determines the cell fate of OPCs. For example, Olig2 localized in the nucleus will induce OPCs to differentiate into myelinating OLs (Zhao, *et al*., 2009). However, cytoplasmic translocation of Olig2 can lead the OPCs to undergo the astrocyte pathway (Cassinani-Ingoni, *et al.*, 2006; Magnus, *et al*., 2007; Zhao, *et al*., 2009). Therefore, we collected cell data separately as $Olig2^{Nuc}$+ve and $Olig2^{Cyto}$+ve, respectively, with OL or astrocyte lineage cell markers (Figure 15).

For immunohistochemistry study, we used double labeling of PDGFαR with Olig2, PDGFαR with GFAP, and Olig2 with GFAP respectively. The PDGFαR+ve/GFAP-ve cell represented glial progenitor (Figure 16C,F); PDGFαR+ve/ $Olig2^{Nuc}$+ve represented OL progenitor (Figure 16B,E); and $Olig2^{Nuc}$+ve/GFAP-ve cells represented OL lineage cells (Figure 16A,D). Instead of comparing the cell densities between ages, the statistical analysis focused on the effects of treatment on the cell densities of these three cell phenotype at a single age. The two-way ANOVA was employed, where the phenotype of the cells and treatment were used as the independent variables.

Histogram depicting the mean number of Hoechst +ve nuclei in the RCC



(n=3)

Immunohistochemical staining depicting the Hoechst +ve nuclei in the RCC

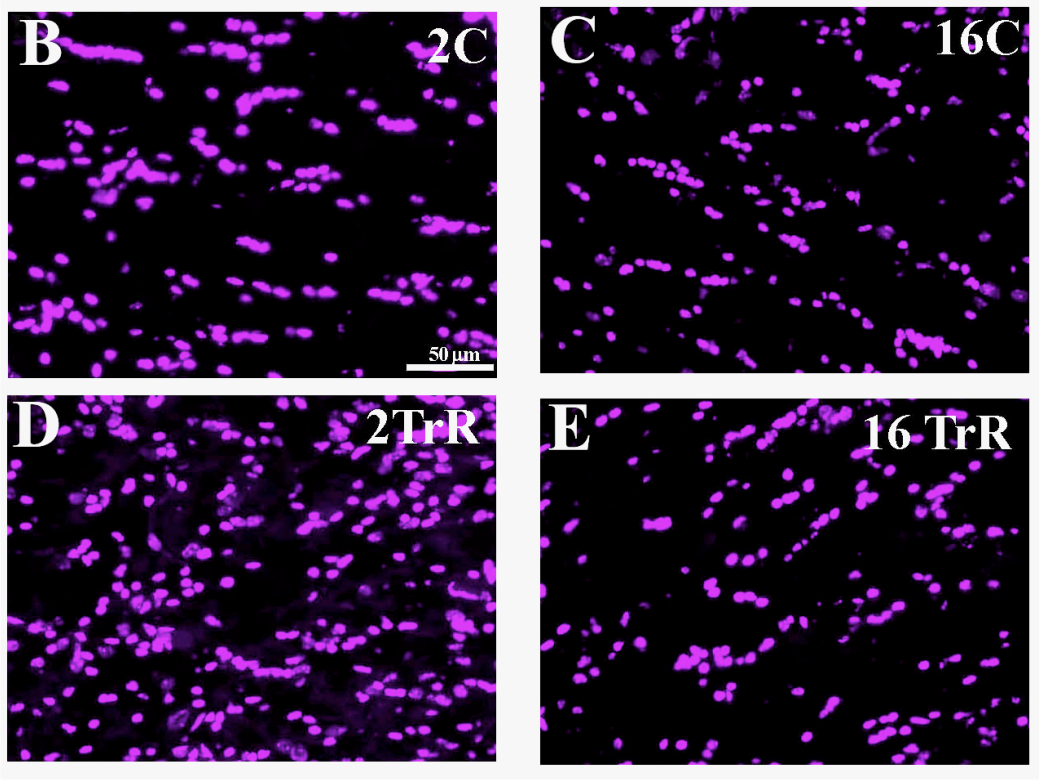


Figure 14. A) Histogram depicting the mean number of Hoechst +ve nuclei in the RCC of 2-16 months old control, cuprizone-treated, and cuprizone recovery mice (n=3). Data revealed that the age and treatment were main effects on the cell densities ($p<0.0001$), as well as the interaction between age and treatment ($p<0.0001$). The brackets denote significant differences (Bonferroni's post test, $p<0.05$). B,C,D,E) Hoechst +ve nuclei in the RCC of 2 months control (B), 16 months control (C), 2 months cuprizone recovery (D), and 16 months cuprizone recovery mice (E). Bar=50 microns.

For cell densities of glial progenitors (PDGFαR+ve/GFAP-ve), a significant 4.2- to 3.1-fold increase after cuprizone treatment (16.0 +/- 3.35) and recovery (11.59 +/- 2.04) was found in the RCC of 2 months old mice, compared to that of 2 months old control mice (3.78 +/- 0.61), respectively ($p<0.5$) (Figure 17A). However, there were no significant differences in glial progenitors at 6, 12, and 16 months cuprizone and cuprizone recovery mice ($p>0.5$) (Figure 17B,C,D). In addition, for OL progenitors (PDGFαR+ve/ $Olig2^{Nuc}$+ve), none of treatment-related differences were observed at any of the ages ($p>0.05$) (Figure 17 A-D).

With respect to OL lineage cells ($Olig2^{Nuc}$+ve/GFAP-ve), Bonferroni's post test revealed that the cell densities were higher in control mice RCC compared to cuprizone-treated and cuprizone recovery mice. For instance, cell number of OL in the 2 month-old control mice (20.27+/-3.05) was 8.2- and 2.3-fold higher than that of cuprizone (2.48+/-0.88) and cuprizone recovery (8.33+/-2.44) mice ($p<0.5$) (Figure 17A). In the RCC of 6 months old mice, cell densities of control mice (16.28+/-3.41) was significantly higher than that of cuprizone mice (5.98+/-3.14) ($p<0.5$) (Figure 17B). Similar to the 2 month-old mice, the cell densities of OL lineage cells were considerably lower after cuprizone treatment and cuprizone recovery (Figure 17C). As well, there was a decline of cell number after cuprizone recovery at 16 month-old mice, compared to the age-matched control (Figure 17D).

Translocation of Olig2 protein determines the cell fate of OPCs

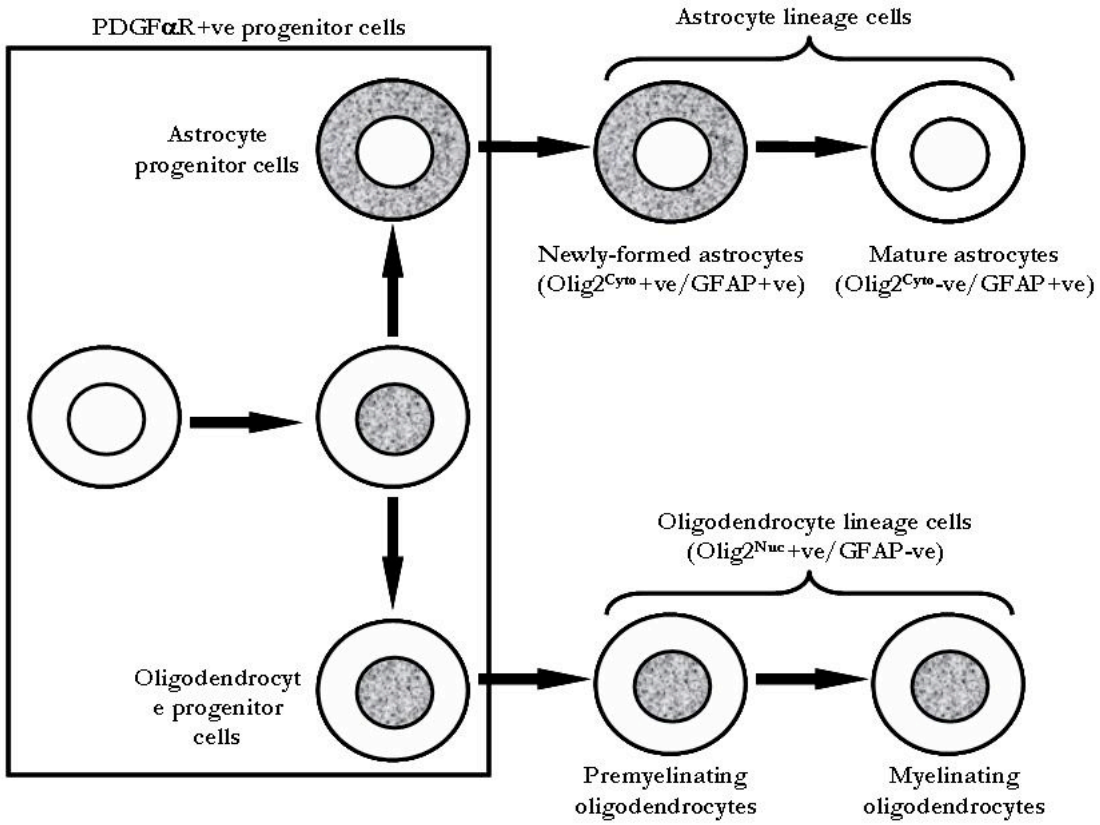


Figure 15. Translocation of Olig2 protein determines the cell fate of OPCs. The gray color represents Olig2 protein in either nucleus or cytoplasm. As the figure shows, Olig2 localized in the nucleus will induce OPCs to differentiate into OL lineage cells. Instead, cytoplasmic translocation of Olig2 can lead the OPCs to undergo the astrocyte pathway.

Immunohistochemical analysis of OL and astrocyte lineage cells

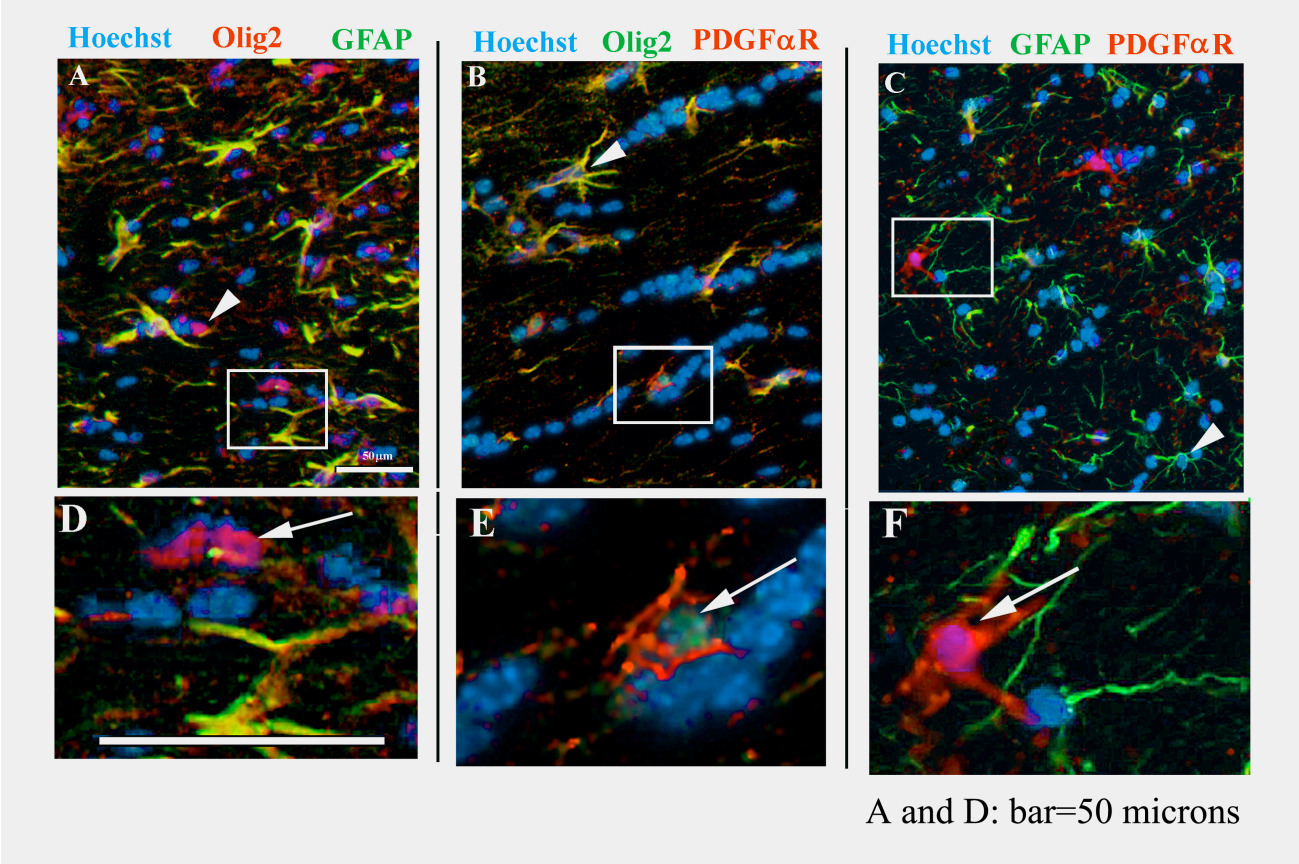


Figure 16. Immunohistochemical analysis of Olig2^{Nuc}+ve/GFAP-ve cells (A, D), Olig2^{Nuc}+ve/PDGFαR+ve cells (B, E), PDGFαR+ve/GFAP-ve cells (C, F) in coronal sections through the RCC (n=3). The images were obtained from CC of 6 month-old cuprizone (A, D), cuprizone recovery (B, E), and control (C, F) mice. The white boxed area in 'A'–'C' denote the portion of each image that is shown at higher magnification in 'D'-'F', respectively. 'D' shows two Olig2^{Nuc}+ve/GFAP-ve cells in the top center of the image (arrow). 'E' shows an Olig2^{Nuc}+ve/PDGFαR+ve cell in the center of the image (arrow). 'F' shows a PDGFαR+ve/GFAP-ve cell on the left side of the image (arrow). The arrowhead in 'A' points out an Olig2^{Cyto}+ve/GFAP-ve cell, in 'B' points out an Olig2^{Cyto}+ve/PDGFαR+ve cell, and in 'C' points out a PDGFαR-ve/GFAP+ve astrocyte. bar (A, D) = 50 microns.

Histograms depicting the mean number of OL lineage cells in the RCC

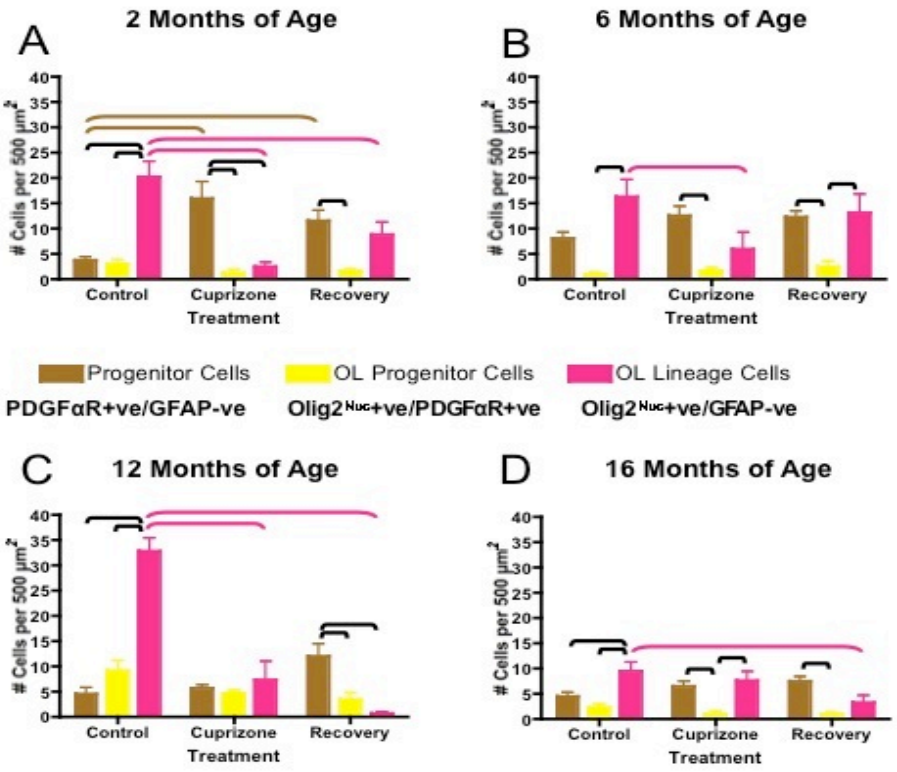


Figure 17. Histograms depicting the mean number (+/- SEM) of progenitor (PDGFαR+ve/GFAP-ve), OL progenitor (Olig2^{Nuc}+ve/PDGFαR+ve), and OL lineage (Olig2^{Nuc}+ve/GFAP-ve) cells in the RCC of 2 (**A**), 6 (**B**), 12 (**C**) and 16 (**D**) month-old control, cuprizone and cuprizone recovery mice (n=3). The two-way ANOVAs showed a significant main effect of phenotype ($p<0.0001$ for all four ages) as well as a significant interaction between phenotype and treatment ($p<0.0001$, $p=0.02$, $p<0.0001$ and $p=0.004$, respectively). There was also a significant main effect of treatment, but only at 12 months of age ($p<0.0001$). For all histograms, the brackets in color denote significant differences within a phenotype (e.g. Olig2^{Nuc}+ve/GFAP-ve), whereas black brackets denote significant differences between phenotypes (Bonferroni's post-test; significance level of $p\leq0.05$).

4.11 Changes in density of the astrocyte lineage after cuprizone-induced demyelination and three weeks recovery

Astrocytes respond to tissue damage, thus any changes in astrocyte cell number after cuprizone or cuprizone recovery could be as result of cuprizone treatment or aging. With regard to astrocyte lineage cell, we focused on the immunostaining of Olig2/GFAP and PDGFαR/Olig2. Due to the functions of cytoplasmic Olig2 protein, we collected data of PDGFαR+ve/ $Olig2^{Cyto}$+ve (Figure 18B) as astrocyte progenitor cells; $Olig2^{Cyto}$+ve/GFAP+ve cells represented new-formed astrocyte (Figure 18A); and $Olig2^{Cyto}$-ve/GFAP+ve cells that signified mature astrocytes. We analyzed the data by two-way ANOVA, as described previously. The independent variables were cell phenotype and the treatment. We focused on the comparison within the same age, instead of within all four ages.

For 2 month-old mice, a huge increase in astrocyte progenitor cells (PDGFαR+ve/ $Olig2^{Cyto}$+ve) was observed after cuprizone treatment (42 fold) and cuprizone recovery (32 fold) (Figure 18A). In addition, the cell number of newly-formed astrocytes was significantly increased after cuprizone treatment compared to the age-matched control (Figure 18A). However, none of difference was found in the cell densities of mature astrocyes (Figure 18A). It suggests that cuprizone treatment affected the cell densities of astrocyte progenitors and newly-formed astrocytes, but not mature astrocytes.

With respect to 6 month-old mice, there was a significant increase in astrocyte progenitors after cuprizone recovery, compared to the age-matched control (Figure 18B). In addition, considerable increases in newly-formed astrocytes were observed after cuprizone treatment and cuprizone recovery (Figure 18B). Moreover, significant increases in cell densities were shown in mature astrocytes after cuprizone treatment and cuprizone recovery (Figure 18B). Thus, cuprizone treatment and cuprizone recovery affected the cell densities in all three cell types of astrocytes.

Similar to the 2 month-old mice, there were significant increases in astrocyte progenitors after cuprizone treatment and cuprizone recovery (Figure 18C). Treatment-related differences were also seen in the cell number of newly formed astrocytes in the RCC of 12 month-old mice (Figure 18C). Compared to that of 6 months of age, there was no statistical difference in mature astrocyte cell density during cuprizone treatment and recovery (Figure 18C). Therefore, treatment-related differences were seen only in astrocyte progenitors and new-formed astrocytes, but not in mature astrocytes.

In the RCC of 16 month-old mice, significant increases in astrocyte progenitors and new-formed astrocytes were only revealed after cuprizone treatment (Figure 18D). There were no considerable changes in cell densities in astrocyte progenitors and newly formed astrocytes after cuprizone recovery at the age ((Figure 18D). In addition, no changes in mature astrocytes were observed, during either cuprizone treatment or cuprizone recovery (Figure 18D). Thus, the cuprizone-related effects on cell density of astrocyte were confined to astrocyte progenitors and newly formed astrocytes.

Histograms depicting the mean number of astrocyte lineage cells in the RCC

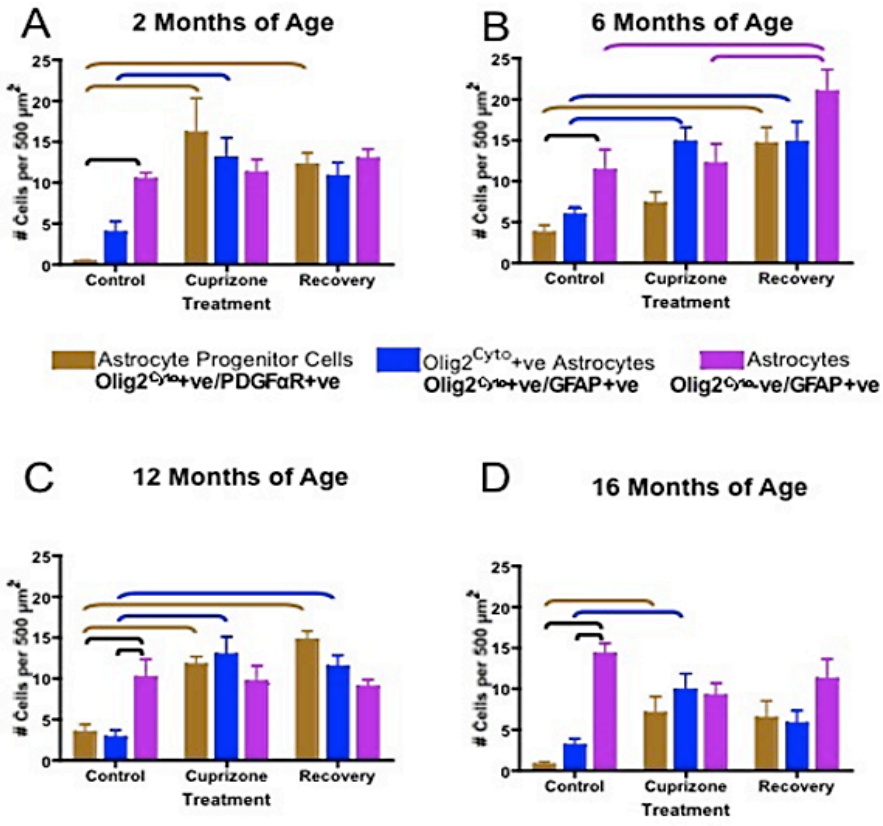


Figure 18. Histograms depicting the mean number of astrocyte progenitor cells, newly formed astrocytes, and mature astrocyte in the RCC of 2 (A), 6 (B), 12 (C), and 16 (D) months old control, cuprizone treatment, and cuprizone recovery mice (n=3). The two-way ANOVA revealed a significant effect of treatment ($p<0.0001$) and interaction ($p=0.009$) in 2 month-old. There was also significant effects of phenotype ($p<0.001$) and interaction ($p<0.0001$) in 6 month-old mice. For 12 month-old mice, there were significant effects of treatment and interaction ($p<0.0001$), but no significant effect of phenotype. For 16 months old mice, there was a main effect of phenotype ($p<0.0001$) and a significant interaction effect ($p=0.003$). For all histograms, the brackets denote significant differences (Bonferroni's post test, $p<0.05$).

V Discussion

The efficiency of OL myelination and remyelination is decreased following aging. Significant research has demonstrated that the reduced efficiency of remyelination is due to the impaired OL progenitor recruitment and differentiation (Ando, *et al.*, 2003; Franklin, *et al*., 2002; Peters, 2002; Rist and Franklin, 2008; Sim, *et al.*, 2002). Furthermore, changes in transcriptional control has also been implicated . Transcriptional factors that up-regulate or prevent myelin gene expression were widely investigated (Franklin, *et al.*, 2002; Sim, *et al.*, 2002). Also, the expression of growth factors known to be important for oligodendrogenesis are delayed in older animals compared to young adults (Franklin, *et al.*, 2002; Hinks and Franklin, 2000). All aspects have the possibility to contribute to impaired OL recruitment and delayed OPCs differentiation.

In this study we focused on the recruitment and differentiation of OL progenitor cells. This research has provided some indication as to why the efficiency of myelination and remyelination were reduced in older animals. First, we showed an age-related reduction in OL progenitor cells recruitment, even though there was no age-related difference in the size of progenitor pool (Figure 14, 17). The cell densities of glial progenitors (PDGFαR+ve/GFAP-ve) and OL progenitor cells (PDGFαR+ve/ $Olig2^{Nuc}$+ve) appeared to remain fairly constant across all 4 ages of control mice. Second, there was no significant increase in glial progenitors and OL progenitors after cuprizone treatment in the adult (6 months) and aged (12, 16 months) mice. In contrast, there were significant increases in cell densities of astrocyte progenitor cells after cuprizone treatment. Third, we found significant increases in cell densities of newly-formed astrocytes ($Olig2^{Cyto}$+ve/GFAP+ve) in 2 to 16 months old mice after cuprizone treatment (Figure 18). In addition, the translocation of Olig2 from nuclei to cytoplasm showed in our data, implies cell fate change after cuprizone treatment in older mice. Fourth, with respect to TFs *Olig2* and *Nkx2.2*, there was an age-related reduction in *Olig2* expression in the control mice, while the expression of *Nkx2.2* was significantly increased after cuprizone

treatment in older aged mice. Finally it would appear that between 12 and 16 months was the time point in aging mice that there is an age-related decrease of OL lineage cells in control mice.

With respect to the reduced myelination efficiency in older animals, we compared the progenitor cell pools within 4 ages of control mice. No significant difference was found in the aged control mice compared to the younger age. In control mice, the cell densities of both glial cell and OL progenitors were fairly constant. Thus, we confirmed that this decreased efficiency is not due to a change in the progenitor pool of these cells. In addition, the density of glial progenitors only increased significantly in 2 months cuprizone mice compared to the age-matched control (Figure 17). This was also confirmed in another study which employed a tamoxifen-inducible Cre-ER under the control of *Olig2* promoter gene as well as a ROSA-EGFP reporter to trace the fate of Olig2+ve cells (Islam, *et al.*, 2009). Furthermore, in mice at 12 and 16 months of age, we found the density of glial progenitors in cuprizone mice was only 36% and 41% ($p<0.05$), respectively, compared to the cell density seen in the 2 month-old cuprizone mice. According to Rivers, *et al*., (2008), the cell cycle of glial progenitors is only about one week. Hence, 6 weeks of cuprizone treatment provided sufficient time to generate glial progenitor cells.

The study of Rivers *et al.* (2008) demonstrated that about one-third of mature OLs present in the RCC of 8.5 month-old mice had been formed *de novo* between 1.5 and 8.5 months of age. Thus, there is accumulation of OLs starting from the younger age until at least 8.5 months of age in mice. From my project, it was not surprising to find that the densities of OL lineage cells in 12 months control mice were significantly higher ($p<0.05$) than that in 2 and 6 month-old mice. However, the cell density were down at 16 months of age. These findings suggest that OLs may continue to generate up to 12 month-old in the RCC of mice, and start losing the cell population sometime prior to 16 months of age. To our knowledge, no one has assessed OL cell numbers past 16 months of age.

In spite of the increased numbers of OL lineage cells that were present in the 12 month-old control mice, there was a 3.1- and 2.1-fold decrease in *MOG* and *CGT* gene expression, respectively, compared to 2 months control. However, *CGT* gene expression did not show a significant difference between 2 and 6 month-old mice. Data suggests that 12 months is likely to be the time point for OL progenitor cells to begin to lose their differentiation capability. Although *MOG* gene expression exhibited significant increases after cuprizone treatment and cuprizone recovery at 12 and 16 month-old mice, immunohistochemical studies failed to show a recovery of myelin in the RCC. It can be speculated that this is probably because the ability of genes to translate into proteins was reduced with age. Combined with the results of decreased number of OL lineage cells after 12 months, It would appear that 12 months is the key point for aged mice to lose the ability of myelination and remyelination.

Data also show that the expression of TFs changed with age. We found that *Nkx2.2* expression showed an age-related increase, whereas Olig2 expression was down-regulated in the control mice. *Olig2* is expressed at all stages of OL lineage cells. The locations of Olig2 direct the cell fate determination of OPCs. Especially, when Olig2 is located in the nucleus, it functions as transcriptional activator to promote the differentiation and maturation of OLs (Nicolay, *et al.*, 2007). On the other hand, Nkx2.2 is believed as a transcriptional repressor in OL lineage cells during normal aging and response to demyelination lesions. Particularly, Nkx2.2 represses genes associated with differentiation and maturation of OLs (Tochitani and Hayashizaki, 2008; Wei, *et al.*, 2005). Researches have indicated that Nkx2.2 TF repress myelin gene expression (Gokhan, *et al.*, 2005; Wei, *et al.*, 2005). In addition, after down-regulation of Nkx2.2, OL lineage cells express a myelinating phenotype (Nicolay, *et al.*, 2004). Furthermore, expression of Nkx2.2 is significantly decreased in terminally differentiated OLs, and the knockdown of Nkx2.2 results in enhanced OL differentiation (Tochitani and Hayashizaki, 2008).

It is possible that increased expression of Nkx2.2 contributes to the fate choice of glial progenitors to differentiate into astrocytes, directly or indirectly. Sun *et al*. (2003) reported there are both cooperative and cross-repressive interactions between Nkx2.2 and Olig2. However, the physical interaction between these two TFs was not sufficient for OL development. Hence, the cross-repressive interaction between these TFs might be altered during normal aging and in response to CNS demyelination in older mice. Therefore, the alteration of this interaction may play a significant role in impeding the OL progenitor cells to respond to the cuprizone treatment in the older mice. Indeed, we found higher expression levels of *Nkx2.2* in 12 and 16 month-old mice.

Our use of the cuprizone model on mice as old as 16 months of ages has demonstrated the tissue of the CC retains the capacity to generate new OLs even in mice as old as 16-month old. However, the cell densities of OL lineage cells are drastically reduced in the RCC of 12 and 16 month-old mice after cuprizone recovery. This is evidence of insufficient generation of new OLs to replace those lost due to the cuprizone treatment. This is likely because the cell fate the OPCs choose to differentiate is into astrocytes and not OLs. The data show an increased percentage of astrocyte progenitors in total glial cell progenitors, as well as in the increased cell densities after cuprizone treatment and cuprizone recovery mice in 12 and 16 month-old mice. Other researchers have demonstrated an increased number of glial progenitors in response to CNS injury (Cassinai-Ingoni, *et al.*, 2006); Magnus, *et al.*, 2007). Data from my thesis shows an increased possibility of Olig2 expression in cytoplasm of glial progenitors in aged mice, allowing these progenitors to differentiate into astrocytes (Setoguchi and Kondo, 2004).

A recent report (Islam, *et al.*, 2009) showed no increase in the percentage of GFAP+ve/GFP+ve cells in the external capsule of 8-10 weeks old cuprizone mice. We also confirmed that there was no significant change in mature astrocytes in the 2 month-old cuprizone mice. We quantified the cell densities of astrocyte lineage cells, including astrocyte progenitors, newly-formed astrocytes, and mature astrocytes. The cell densities

of newly-formed astrocytes were higher in the RCC of mice after cuprizone treatment, compared to the age-matched control. In addition, there were also more astrocyte progenitors in the RCC in response to cuprizone-induced demyelination.

In conclusion, our findings provide some insight into why there is an age-related decreased efficiency of myelination and remyelination. The size of progenitor cell pool seems to remain fairly constant up to 16 months of age. However, the cells preferably differentiate into astrocyte lineage as opposed to OL lineage cell, starting at 6 months of age. TFs Nkx2.2 and Olig2 may be involved in this progress. The age-related increase of *Nkx2.2* gene expression would certainly impede the differentiation of progenitors into OL lineage cells. In addition, the cytoplasmic translocation of *Olig2* TF would facilitate their differentiation into astrocytes. Hence, a first step in improving remyelination efficiency in older animals may require both a reversal of age-related increased expression of transcriptional repressor Nkx2.2 and preventing the cytoplasmic translocation of Olig2.

VI Future directions

In the present work, I investigated expression levels of myelin-related genes as well as some critical TFs, which are involved in OL lineage cells' differentiation and maturation. The data collected from immunohistochemistry and qRT-PCR is useful for interpreting the changes in OL proliferation and differentiation during aging, as well as providing clues to functions of OL-specific TFs involved in the cell fate determination of OPCs. Our studies showed that 12 months of age in mice appears to be a critical time when mice are capable of remyelination following a demyelinating lesion after which the remyelinating ability declines. In mice of 12 months of age, MOG and MBP immunostaining after cuprizone recovery did not show as good a recovery as mice of younger age. As well, the gene expression levels of *Olig2* and *Nkx2.2* showed significant differences, beginning at 12 months of age. It is possible that the changes in gene expression of TFs may affect recruitment of OPCs from the glial progenitor pool. In addition, gene expression level of *PDGF α R* also showed a significant increase after cuprizone treatment only in the 12 month aged mice, indicating that an age related change in responsiveness is occurring then. Consequently, the complex interaction between OL-specific genes and TFs will be an important area of further study especially in the context of aging. Gain-of -function or loss-of-function approach may help to investigate the main effectors of aging.

My findings implicate TFs Olig2 and Nkx2.2 as important components of the transcriptional control network that may contribute to the age-related switch in fate choice of the PDGF α R+ve progenitor cells into OLs. Hence, control of gene expression of the TFs Nkx2.2 and Olig2 would be the first step in improving the differentiation of those progenitor cells into OLs, and therefore limit the extent of demyelination that can occur during normal aging and indeed improve remyelination efficiency in older animals. Sun, *et al*., (2003) reported that there are both cooperative and cross-repressive interactions between Nkx2.2 and Olig2, with the protein-protein interaction depending on

the homeodomain and the bHLH domain of each TF respectively. However, Sun *et al*., (2003) demonstrated the physical interaction between the two TFs was not sufficient for OL development, although it is possible that the cross-repressive interaction between two TFs may be altered during normal aging and in response to CNS demyelination in the older animals. If this were the case, then this altered interaction could play a significant role in impeding the OPCs response to normal aging and to cuprizone treatment. For further studies, both *in vivo* and *in vitro* experiments could be used to test the interaction between Olig2 and Nkx2.2. CG4 cell is one of the cell lines that is widely used to investigate the OL development and differentiation *in vitro* (Todorich, *et al.*, 2008). Vectors carrying Nkx2.2 or Olig2 genes can be used to upregulate expression or iRNA can be used to down-regulate Nkx2.2 or Olig2 to determine what role the TFs have on CG4 oligodendroglial differentiation and development. These experiments would also be used to determine impact of manipulating expression of one TF such Olig2 and measuring its effect on Nkx2.2 and vice versa to determine if relationship existed between these genes during OL differentiation or in OL cell fate determination. For the *in vivo* study a traditional gene knock-out strategy using homologous recombination can be used if the loss-of function of the gene is not lethal. However the Cre/*lox*p strategy may be better suited if a controlled conditional deletion of the gene is required in cases when the deletion of gene is lethal as is the case for Olig2 (Ono, *et al.*, 2008; Masahira, *et al.*, 2006). In the Cre/*loxp* strategy, the gene of interest is flanked by *loxp* sites which are recognized by the enzyme cre and in a controlled manner can be used to delete the gene between the loxp sites at any time in the aging animal after treatment with a drug such as tamoxifen that can induce this process (Ono, *et al.*, 2008; Masahira, *et al.*, 2006). Cre/*loxp* strategy would be a good way to investigate the interaction between Olig2 and Nkx2.2 in adult aging mice *in vivo*.

My findings also reveal the possibility of OPC differentiation into astrocytes in older animals and can contribute to further investigations in diseases, such as MS and AD

(Connor, 2004; Kahle, *et al.*, 2009; Lindsberg, *et al.*, 2010).The ratio of OLs *vs*. astrocytes may provide clues to the mechanism(s) involved in these diseases. The increased ratio of astrocytes to OL may induce mental diseases (section 2.3). In future studies that focus on those diseases it may be possible in animal models to compare the cell density of astrocytes between young and old animals.

VII References

Ahlenius H, Visan V, Kokaia M, Lindvall O, Kokaia Z. (2009) Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. J Neurosci. 29(14):4408-19.

Ahlgren SC, Wallace H, Bishop J, Neophytou C, Raff MC. (1997) Effects of thyroid hormone on embryonic oligodendrocyte precursor cell development in vivo and in vitro. Mol Cell Neurosci. 9(5-6):420-32.

Alberta JA, Park S-K, Mora J, Yuk D, Pawlitzky I, Iannarelli P, Vartanian T, Stiles CD, Rowitch DH. (2001) Sonic hedgehog is required during an early phase of oligodendrocyte development in mammalian brain. Mol Cell Neurosci 18:434 – 441.

Albrecht J, Sonnewald U, Waagepetersen HS, Schousboe A. (2007) Glutamine in the central nervous system: function and dysfunction. Front Biosci. 12:332-43.

Almazan G, McKay R. (1992) An oligodendrocyte precursor cell line from rat optic nerve. Brain Res. 579(2):234-45.

Antony JM, van Marle G, Opii W, Butterfield DA, Mallet F, Yong VW, Wallace JL, Deacon RM, Warren K, Power C. (2004) Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. Nat Neurosci. 7(10):1088-95. Epub 2004 Sep 26.

Anderson TJ, Schneider A, Barrie JA, Klugmann M, McCulloch MC, Kirkham D, Kyriakides E, Nave KA, Griffiths IR. (1998) Late-onset neurodegeneration in mice with increased dosage of the proteolipid protein gene. J Comp Neurol. 394(4):506-19.

Ando, S, Tanaka, Y, Toyoda, Y, and Kon, K. (2003) Turnover of myelin lipids in aging brain. Neurochem Res. 28(1): p. 5-13.

Armstrong, RC., Le, TQ., Frost, EE., Borke, RC., Vana, AC. (2002) Absence of fibroblast growth factor 2 promotes oligodendroglial repopulation of demyelinated white matter. J Neurosci. 22(19): p. 8574-85.

Arnett, HA, Fancy, S, Alberta, J, Zhao, C, Plant, S, Kaing, S, Raine, C.S, Rowitch, DH, Franklin, RJ, and Stiles, CD. (2004) bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. Science, 306: p. 2111-2115.

Aremu DA, Meshitsuka S. (2006) Some aspects of astroglial functions and aluminum implications for neurodegeneration. Brain Res Rev. 52(1):193-200.

Artemiadis AK, Anagnostouli MC. (2010) Apoptosis of oligodendrocytes and post-translational modifications of myelin basic protein in multiple sclerosis: possible role for the early stages of multiple sclerosis. Eur Neurol. 63(2):65-72.

Antony JM, van Marle G, Opii W, Butterfield DA, Mallet F, Yong VW, Wallace JL, Deacon RM, Warren K, Power C. (2004) Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. Nat Neurosci. 7(10):1021-3.

Awatramani R, Scherer S, Grinspan J, Collarini E, Skoff R, O'Hagan D, Garbern J, Kamholz J. (1997) Evidence that the homeodomain protein Gtx is involved in the regulation of oligodendrocyte myelination. J Neurosci 17:6657–6668.

Baracskay KL, Kidd GJ, Miller RH, Trapp BD. (2007) NG2-positive cells generate A2B5-positive oligodendrocyte precursor cells. Glia. 55(10):1001-10.

Barres BA. (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron. 60(3):430-40. Review.

Barres BA, Lazar MA, Raff MC. (1994) A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development. 120(5):1097-108.

Bartzokis, G. (2004) Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiol Aging, 25(1): p. 5-18.

Bartzokis, G, Sultzer, D, Lu, PH, Nuechterlein, KH, Mintz, J, and Cummings, JL. (2004) Heterogeneous age-related breakdown of white matter structural integrity: implications for cortical "disconnection" in aging and Alzheimer's disease. Neurobiol Aging, 25(7): p. 843-51.

Bauer NG, Richter-Landsberg C, Ffrench-Constant C. (2009) Role of the oligodendroglial cytoskeleton in differentiation and myelination. Glia. 2009 May 19. [Epub ahead of print].

Baumann N, Pham-Dinh D. (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev. 81(2):871-927.

Berlet, HH. and Volk, B. (1980) Studies of human myelin proteins during old age. Mech Ageing Dev,14(1-2): p. 211-22.

Bernstein HG, Steiner J, Bogerts B. (2009) Glial cells in schizophrenia: pathophysiological significance and possible consequences for therapy. Expert Rev Neurother. 9(7):1059-71. Review.

Biegon, A, Eberling, JL (1994). Human corpus callosum in aging and Alzheimer's disease: a magnetic resonance imaging study. Neurobiology of aging 15(4): 393-397.

Blakemore WF, Keirstead HS. (1999) The origin of remyelinating cells in the central nervous system. J Neuroimmunol. 98(1):69-76.

Boggs JM, Rangaraj G. (2000) Interaction of lipid-bound myelin basic protein with actin filaments and calmodulin. Biochemistry. 39(26):7799-806.

Booth J, Nicolay DJ, Doucette JR, Nazarali AJ. (2007) Hoxd1 is expressed by oligodendroglial cells and binds to a region of the human myelin oligodendrocyte glycoprotein promoter in vitro. Cell Mol Neurobiol. 27(5):641-50.

Bosio A, Binczek E, Stoffel W.(1996) Molecular cloning and characterization of the mouse CGT gene encoding UDP-galactose ceramide-galactosyltransferase (cerebroside synthetase). Genomics. 35(1):223-6.

Braak H, Del Tredici K, Schultz C, Braak E. (2000) Vulnerability of select neuronal types to Alzheimer's disease. Ann N Y Acad Sci. 2000;924:53-61.

Bradl M, Linington C. (1996) Animal models of demyelination. Brain Pathol. 6(3):303-11.

Breteler MM, Claus JJ, van Duijn CM, Launer LJ, Hofman A. (1992) Epidemiology of Alzheimer's disease. Epidemiol Rev. 1992;14:59-82. Review.

Briscoe J, Pierani A, Jessell TM, Ericson J. (2000) A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell

101:435 – 445.

Bronstein JM, Micevych PE, Chen K. (1997) Oligodendrocyte-specific protein (OSP) is a major component of CNS myelin. J Neurosci Res. 50(5):713-20.

Brunner C, Lassmann H, Waehneldt TV, Matthieu JM, Linington C. (1989) Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase in the CNS of adult rats. J Neurochem. 52(1):296-304.

Butt AM, Pugh M, Hubbard P, James G. (2004) Functions of optic nerve glia: axoglial signalling in physiology and pathology. Eye (Lond). 18(11):1110-21. Review.

Butts BD, Houde C, Mehmet H. (2008) Maturation-dependent sensitivity of oligodendrocyte lineage cells to apoptosis: implications for normal development and disease. Cell Death Differ. 15(7):1178-86.

Byravan S, Foster LM, Phan T, Verity AN, Campagnoni AT. (1994) Murine oligodendroglial cells express nerve growth factor. Proc Natl Acad Sci U S A. 91(19):8812-6.

Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD. (1998) Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron. 20(5):869-82.

Carlson NG, Rojas MA, Redd JW, Tang P, Wood B, Hill KE, Rose JW. (2010) Cyclooxygenase-2 expression in oligodendrocytes increases sensitivity to excitotoxic death. J Neuroinflammation. 13;7:25.

Cassiani-Ingoni, R, Coksaygan, T, Xue, H, Reichert-Scrivner, S.A, Wiendl, H, Rao, M.S, Magnus, T. (2006). Cytoplasmic translocation of Olig2 in adult glial progenitors marks the generation of reactive astrocytes following autoimmune inflammation. Exp. Neurol. 201:349-358.

Chakraborty G, Ziemba S, Drivas A, Ledeen RW. (1997) Myelin contains neutral sphingomyelinase activity that is stimulated by tumor necrosis factor-alpha. J Neurosci Res. 50(3):466-76.

Chen CS, Johnson MA, Flower RA, Slater BJ, Miller NR, Bernstein SL. (2008) A primate model of nonarteritic anterior ischemic optic neuropathy. Invest Ophthalmol Vis Sci. 49(7):2985-92.

Chisari M, Shu HJ, Taylor A, Steinbach JH, Zorumski CF, Mennerick S. (2010) Structurally diverse amphiphiles exhibit biphasic modulation of GABAA receptors: similarities and differences with neurosteroid actions. Br J Pharmacol. 160(1):130-41.

Chiti A, Giorli E, Gallerini S, Morelli N, Orlandi G. (2009) Stroke and age-brain barrier: how many bricks in the wall? Acta Neurol Belg. 2009 Mar;109(1):7-9. Review.

Chittajallu R, Aguirre A, Gallo V. (2004) NG2-positive cells in the mouse white and grey matter display distinct physiological properties. J Physiol. 15;561(Pt 1):109-22.

Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, Suzuki K, Popko B.(1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell. 86(2):209-19.

Coffey JC, McDermott KW. (1997) The regional distribution of myelin oligodendrocyte glycoprotein (MOG) in the developing rat CNS: an in vivo immunohistochemical study. J Neurocytol. 26(3):149-61.

Collarini, EJ, Pringle, N, Mudhar, H, Stevens, G, Kuhn, R, Monuki, ES, Lemke, G, and Richardson, WD. (1991) Growth factors and transcription factors in oligodendrocyte development. J Cell Sci Suppl, 15: p. 117-23.

Connor, JR. (2004). Myelin breakdown in Alzheimer's disease: a commentary. Neurobiol Aging 25(1): 45-47.

Connor JR, Menzies SL. (1996) Relationship of iron to oligodendrocytes and myelination. Glia. 17(2):83-93. Review.

Dai X, Lercher LD, Yang L, Shen M, Black IB, Dreyfus, CF. (1997) Expression of neurotrophins by basal forebrain (BF) oligodendrocytes. Soc Neurosci Abstr. 23, 331.

Dawson MR, Levine JM, Reynolds R. (2000) NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res. 61(5):471-9. Review.

Deneen B, Ho R, Lukaszewicz A, Hochstim CJ, Gronostajski RM, Anderson DJ. (2006) The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. Neuron 52:953–968.

Dickinson PJ, Fanarraga ML, Griffiths IR, Barrie JM, Kyriakides E, Montague P. (1996) Oligodendrocyte progenitors in the embryonic spinal cord express DM-20. Neuropathol Appl Neurobiol. 22(3):188-98.

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Götz M. (2008) Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci. 28(41):10434-42.

Doetsch F, García-Verdugo JM, Alvarez-Buylla A. (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci. 17(13):5046-61.

Doucette JR, Jiao R, Nazarali AJ. (2010) Age-related and cuprizone-induced changes in myelin and transcription factor gene expression and in oligodendrocyte cell densities in the rostral corpus callosum of mice. Cell Mol Neurobiol. 30(4):607-29.

Dratviman-Storobinsky O, Hasanreisoglu M, Offen D, Barhum Y, Weinberger D, Goldenberg-Cohen N. (2008) Progressive damage along the optic nerve following induction of crush injury or rodent anterior ischemic optic neuropathy in transgenic mice. Mol Vis. 14:2171-9.

Dupree JL, Suzuki K, Popko B. (1998) Galactolipids in the formation and function of the myelin sheath. Microsc Res Tech. 41(5):431-40.

Fernandez M, Pirondi S, Manservigi M, Giardino L, Calzà L. (2004) Thyroid hormone participates in the regulation of neural stem cells and oligodendrocyte precursor cells in the central nervous system of adult rat. Eur J Neurosci. 20(8):2059-70.

Fok-Seang J, Miller RH. (1994) Distribution and differentiation of A2B5+ glial precursors in the developing rat spinal cord. J Neurosci Res. 37(2):219-35.

Franklin RJ, Ffrench-Constant C. (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci. 9(11):839-55. Review.

Franklin RJ, Zhao C, and Sim FJ. (2002) Ageing and CNS remyelination. Neuroreport, 13(7): p. 923-8.

Fu H, Cai J, Rutledge M, Hu X, Qiu M. (2003) Oligodendrocytes can be generated from the local ventricular and subventricular zones of embryonic chicken midbrain. Brain Res Dev Brain Res.;143(2):161-5.

Fu H, Qi Y, Tan M, Cai J, Takebayashi H, Nakafuku M, Richardson W, Qiu M. (2002) Dual origin of spinal oligodendrocyte progenitors and evidence for the cooperative role of Olig2 and Nkx2.2 in the control of oligodendrocyte differentiation. Development. 129(3):681-93.

Fujimoto M, Takagi Y, Muraki K, Nozaki K, Yamamoto N, Tsuji M, Hashimoto N, Honjo T, Tanigaki K. (2009) RBP-J promotes neuronal differentiation and inhibits

oligodendroglial development in adult neurogenesis. Dev Biol. 2009 Jun 4. [Epub ahead of print].

Gallo V, Armstrong RC. (2008) Myelin repair strategies: a cellular view. Curr Opin Neurol. 21(3):278-83. Review.

Gao FB, Apperly J, Raff M. (1998) Cell-intrinsic timers and thyroid hormone regulate the probability of cell-cycle withdrawal and differentiation of oligodendrocyte precursor cells. Dev Biol. 197(1):54-66.

Gandhi R, Laroni A, Weiner HL. (2010) Role of the innate immune system in the pathogenesis of multiple sclerosis. J Neuroimmunol. 15;221(1-2):7-14. Review.

Gard AL, Pfeiffer SE. (1990) Two proliferative stages of the oligodendrocyte lineage (A2B5+O4- and O4+GalC-) under different mitogenic control. Neuron. 5(5):615-25.

Gay F. (2007) Activated microglia in primary MS lesions: defenders or aggressors? Int MS J. 14(3):78-83. Review.

Genoud S, Lappe-Siefke C, Goebbels S, Radtke F, Aguet M, Scherer SS, Suter U, Nave K-A, Mantei N. (2002) Notch1 control of oligodendrocyte differentiation in the spinal cord. J Cell Biol 158:709 – 718.

Giffard RG, Swanson RA. (2005) Ischemia-induced programmed cell death in astrocytes. Glia. 50(4):299-306. Review.

Goldberg JL, Vargas ME, Wang JT, Mandemakers W, Oster SF, Sretavan DW, Barres BA. (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci. 24(21):4989-99.

Goldenberg-Cohen N, Guo Y, Margolis F, Cohen Y, Miller NR, Bernstein SL. (2005) Oligodendrocyte dysfunction after induction of experimental anterior optic nerve ischemia. Invest Ophthalmol Vis Sci. 46(8):2716-25.

Gokhan S, Marin-Husstege M, Yung SY, Fontanez, D, Casaccia-Bonnefil, P, Mehler M. (2005). Combinatorial profiles of oligodendrocyte-selective classes of transcriptional regulators differentially modulate myelin basic protein gene expression. J. Neurosci. 25:8311-8321.

Gootjes, L., A. Bouma. (2006). Corpus callosum size correlates with asymmetric performance on a dichotic listening task in healthy aging but not in Alzheimer's disease. Neuropsychologia 44(2): 208-217.

Goslin K, Banker G. (1990) Rapid changes in the distribution of GAP-43 correlate with the expression of neuronal polarity during normal development and under experimental conditions. J Cell Biol. 110(4):1319-31.

Gravel M, Peterson J, Yong VW, Kottis V, Trapp B, Braun PE. (1996) Overexpression of 2',3'-cyclic nucleotide 3'-phosphodiesterase in transgenic mice alters oligodendrocyte development and produces aberrant myelination. Mol Cell Neurosci. (6):453-66.

Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA. (1996) Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron. 17(4):595-606.

Guillemot F. (2007) Cell fate specification in the mammalian telencephalon. Prog Neurobiol. 83(1):37-52.

Hao Z, Yeung J, Wolf L, Doucette R, and Nazarali A. (1999) Differential expression of Hoxa-2 protein along the dorsal-ventral axis of the developing and adult mouse spinal cord. Dev Dyn, 216(2): p. 201-17.

Harada T, Harada C. (2004) Function of glial cell network as a modulator of neural cell death during retinal degeneration. Nippon Ganka Gakkai Zasshi. 108(11):674-81. Review.

Hardy RJ, Friedrich VL Jr. (1996) Oligodendrocyte progenitors are generated throughout the embryonic mouse brain, but differentiate in restricted foci. Development. 122(7):2059-69.

Henderson AP, Barnett MH, Parratt JD, Prineas JW. (2009) Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol. 66(6):739-53.

Hinks GL, Franklin RJ. (2000) Delayed changes in growth factor gene expression during slow remyelination in the CNS of aged rats. Mol Cell Neurosci, 16(5): p. 542-56.

Ho MS, Chen H, Chen M, Jacques C, Giangrande A, Chien CT. (2008) Gcm protein degradation suppresses proliferation of glial progenitors. Proc Natl Acad Sci USA. 106(16):6778-83.

Ihrie RA, Alvarez-Buylla A. (2008) Cells in the astroglial lineage are neural stem cells. Cell Tissue Res. 331(1):179-91.

Imbimbo BP. (2009) An update on the efficacy of non-steroidal anti-inflammatory drugs in Alzheimer's disease. Expert Opin Investig Drugs. 18(8):1147-68. Review.

Inoue Y, Kagawa T, Matsumura Y, Ikenaka K, Mikoshiba K. (1996) Cell death of oligodendrocytes or demyelination induced by overexpression of proteolipid protein depending on expressed gene dosage. Neurosci Res. 25(2):161-72.

Islam MS, Tatsumi K, Okuda H, Shiosaka S, Wanaka A. (2009) Olig2-expressing progenitor cells preferentially differentiate into oligodendrocytes in cuprizone-induced demyelinated lesions. Neurochem Int. 54(3-4):192-8.

Iwamoto K, Bundo M, Yamada K, Takao H, Iwayama Y, Yoshikawa T, Kato. (2006) A family-based and case-control association study of SOX10 in schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 141B(5):477.

Juliet PA, Frost EE, Balasubramaniam J, Del Bigio MR. (2009) Toxic effect of blood components on perinatal rat subventricular zone cells and oligodendrocyte precursor cell proliferation, differentiation and migration in culture. J Neurochem. 109(5):1285-99.

Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K. (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron. 13(2):427-42.

Kagawa T, Wada T, Ikenaka K. (2001) Regulation of oligodendrocyte development. Microsc Res Tech. 15;52(6):740-5.

Kahle KT, Simard JM, Staley KJ, Nahed BV, Jones PS, Sun D. (2009) Molecular mechanisms of ischemic cerebral edema: role of electroneutral ion transport. Physiology (Bethesda). 24:257-65. Review.

Káradóttir R, Hamilton NB, Bakiri Y, Attwell D. (2008) Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat Neurosci. 11(4):450-6.

Kaur C, Foulds WS, Ling EA. (2008) Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. Prog Retin Eye Res. 27(6):622-47.

Kaur G, Han SJ, Yang I, Crane C. (2010) Microglia and central nervous system immunity. Neurosurg Clin N Am. 21(1):43-51. Review.

Keyser DO, Pellmar TC. (1997) Regional differences in glial cell modulation of synaptic transmission. Hippocampus. 7(1):73-7.

Kiernan BW, Ffrench-Constant C. (1993) Oligodendrocyte precursor (O-2A progenitor cell) migration; a model system for the study of cell migration in the developing central nervous system. Dev Suppl. 219-25. Review.

Kirschner DA, Ganser AL. (1980) Compact myelin exists in the absence of basic protein in the shiverer mutant mouse. Nature. 283(5743):207-10.

Klugmann M, Schwab MH, Pühlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA. (1997) Assembly of CNS myelin in the absence of proteolipid protein. Neuron. 18(1):59-70.

Komitova M, Zhu X, Serwanski DR, Nishiyama A. (2009) NG2 cells are distinct from neurogenic cells in the postnatal mouse subventricular zone. J Comp Neurol. 512(5):702-16.

Komoly S, Hudson LD, Webster, H.D, and Bondy CA. (1984) Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. Proc Natl Acad Sci U S A, 89(5): p. 1894-8.

Kondo A, Nakano T, Suzuki K. (1987). Blood-brain barrier permeability to horseradish peroxidase in twitcher and cuprizone-intoxicated mice. Brain Res 425:186-190.

Kondo T, Raff M. (2000) The Id4 HLH protein and the timing of oligodendrocyte differentiation. EMBO J 19:1998 – 2007.

Kooi EJ, van Horssen J, Witte ME, Amor S, Bø L, Dijkstra CD, van der Valk P, Geurts JJ. (2009) Abundant extracellular myelin in the meninges of patients with multiple sclerosis. Neuropathol Appl Neurobiol. 35(3):283-95.

Kimura M, Sato M, Akatsuka A, Saito S, Ando K, Yokoyama M, Katsuki M. (1998) Overexpression of a minor component of myelin basic protein isoform (17.2 kDa) can restore myelinogenesis in transgenic shiverer mice. Brain Res. 785(2):245-52.

Kimura M, Sato M, Akatsuka A, Nozawa-Kimura S, Takahashi R, Yokoyama M, Nomura T, Katsuki M. (1989) Restoration of myelin formation by a single type of myelin basic protein in transgenic shiverer mice. Proc Natl Acad Sci USA. 86(14):5661-5.

Kuhn TB, Brown MD, Wilcox CL, Raper JA, Bamburg JR. (1999) Myelin and collapsin-1 induce motor neuron growth cone collapse through different pathways: inhibition of collapse by opposing mutants of rac1. J Neurosci. 19(6):1965-75.

Kurz H, Christ B. (1998) Embryonic CNS macrophages and microglia do not stem from circulating, but from extravascular precursors. Glia. 22(1):98-102.

Kwon YK. (2002) Effect of neurotrophic factors on neuronal stem cell death. J Biochem Mol Biol. 35(1):87-93. Review.

Lang W, Rodriguez M, Lennon VA, and Lampert PW. (1984) Demyelination and remyelination in murine viral encephalomyelitis. Ann N Y Acad Sci, 436: p. 98-102.

Le Bras B, Chatzopoulou E, Heydon K, Martínez S, Ikenaka K, Prestoz L, Spassky N, Zalc B, Thomas JL. (2005) Oligodendrocyte development in the embryonic brain: the contribution of the plp lineage. Int J Dev Biol. 49(2-3):209-20.

Lee E, Son H (2009) Adult hippocampal neurogenesis and related neurotrophic factors. BMB Rep. 2009 42(5):239-44.

Lepore AC, Han SS, Tyler-Polsz CJ, Cai J, Rao MS, Fischer I. (2004) Differential fate of multipotent and lineage-restricted neural precursors following transplantation into the adult CNS. Neuron Glia Biol. 1(2):113-126.

Levine JM, Stincone F, Lee YS. (1993) Development and differentiation of glial precursor cells in the rat cerebellum. Glia. 7(4):307-21.

Levison SW, Druckman SK, Young GM, Basu A. (2003) Neural stem cells in the subventricular zone are a source of astrocytes and oligodendrocytes, but not microglia. Dev Neurosci. 25(2-4):184-96.

Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. (2010) Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage. J Cereb Blood Flow Metab. 30(4):689-702.

Linington C, Bradl M, Lassmann H, Brunner C, Vass K. (1988) Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal

antibodies directed against a myelin/oligodendrocyte glycoprotein. Am J Pathol. 130(3):443-54.

Liu A, Li J, Marin-Husstege M, Kageyama R, Fan Y, Gelinas C, Casaccia- Bonnefil P. (2006) A molecular insight of Hes5-dependent inhibiinhibition of myelin gene expression: Old partners and new players. EMBO J 25:4833 – 4842.

Lopez JC. (2001) A beta, tau and the fabrication of tangles. Nat. Rev. Neurosci. 2 p. 681.

Lopes-Cardozo M, Sykes JE, Van der Pal, RH, and van Golde, LM. (1989) Development of oligodendrocytes. Studies of rat glial cells cultured in chemically-defined medium. J Dev Physiol, 12(3): p. 117-27.

Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH. (2002) Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 109:75–86.

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. (2000) Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron 25:317–329.

Lu ZY, Yu SP, Wei JF, Wei L. (2006) Age-related neural degeneration in nuclear-factor kappaB p50 knockout mice. Neuroscience.139(3):965-78.

Ludwin SK. (1979) The perineuronal satellite oligodendrocyte. A role in remyelination. Acta Neuropathol. 47(1):49-53.

Luskin MB, Pearlman AL, Sanes JR. (1988) Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. Neuron. 1(8):635-47.

Mabie PC, Mehler MF, Kessler JA. (1999) Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. J Neurosci 19:7077 – 7088.

Magnus T, Coksaygan T, Korn T, Xue H, Arumugam TV, Mughal MR, Eckley DM, Tang SC, DeTolla L, Rao MS, Cassiani-Ingoni R, Mattson MP. (2007). Evidence that nucleocytoplasmic Olig2 translocation mediates brain-injury-induced differentiation of glial precursors to astrocytes. J Neurosci Res 85:2126-2137.

Martini R, Mohajeri MH, Kasper S, Giese KP, Schachner M. (1995) Mice doubly deficient in the genes for P0 and myelin basic protein show that both proteins contribute to the formation of the major dense line in peripheral nerve myelin. J Neurosci. 15(6):4488-95.

Maeno N, Takahashi N, Saito S, Ji X, Ishihara R, Aoyama N, Branko A, Miura H, Ikeda M, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Iwata N, Inada T, Ozaki N. (2007) Association of SOX10 with schizophrenia in the Japanese population. Psychiatr Genet. 17(4):227-31.

Masahira N, Takebayashi H, Ono K, Watanabe K, Ding L, Furusho M, Ogawa Y, Nabeshima Y, Alvarez-Buylla A, Shimizu K, Ikenaka K. (2006) Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. Dev Biol. 293(2):358-69.

Matsubayashi Y, Iwai L, Toda T, Lu QR, Kawasaki H. (2009) Immunostaining for oligodendrocyte-specific galactosphingolipids in fixed brain sections using the cholesterol-selective detergent digitonin. J Neurosci Methods. 178(1):87-98.

Matsushima GK, and Morell P. (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol, 11(1): p. 107-16.

McCombe PA. (2008) Recombinant EPF/chaperonin 10 promotes the survival of O4-positive pro-oligodendrocytes prepared from neonatal rat brain. Cell Stress Chaperones. 13(4):467-74.

McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE. (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron.;13(4):805-11.

McMahon SS, McDermott KW. (2001) Proliferation and migration of glial precursor cells in the developing rat spinal cord. J Neurocytol. 30(9-10):821-8.

McQuaid S, Cunnea P, McMahon J, Fitzgerald U. (2009) The effects of blood-brain barrier disruption on glial cell function in multiple sclerosis. Biochem Soc Trans. 37(Pt 1):329-31. Review.

Meyer NP, Roelink H. (2003) The amino-terminal region of Gli3 antagonizes the Shh response and acts in dorsoventral fate specification in the developing spinal cord. Dev Biol 257:343 – 355.

Mi S, Sandrock A, Miller RH. (2008) LINGO-1 and its role in CNS repair. Int J Biochem Cell Biol. 40(10):1971-8.

Miller RH. (2002) Regulation of oligodendrocyte development in the vertebrate CNS. Prog Neurobiol. 67(6):451-67.

Mitkus SN, Hyde TM, Vakkalanka R, Kolachana B, Weinberger DR, Kleinman JE, Lipska. (2007) Expression of oligodendrocyte-associated genes in dorsolateral prefrontal cortex of patients with schizophrenia. Schizophr Res. 98(1-3):129-38.

Mizuno T, Kurotani T, Komatsu Y, Kawanokuchi J, Kato H, Mitsuma N, Suzumura A. (2004) Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. Neuropharmacology. 46(3):404-11.

Mokhtarian F, Zhang Z, Shi Y, Gonzales E, Sobel RA. (1999) Molecular mimicry between a viral peptide and a myelin oligodendrocyte glycoprotein peptide induces autoimmune demyelinating disease in mice. J Neuroimmunol. 95(1-2):43-54.

Montag D, Giese KP, Bartsch U, Martini R, Lang Y, Blüthmann H, Karthigasan J, Kirschner DA, Wintergerst ES, Nave KA. (1994) Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. Neuron. 13(1):229-46.

Montague P, Dickinson PJ, McCallion AS, Stewart GJ, Savioz A, Davies RW, Kennedy PG, Griffiths IR. (1997) Developmental expression of the murine Mobp gene. J Neurosci Res. 49(2):133-43.

Morath DJ, Mayer-Pröschel M. (2001) Iron modulates the differentiation of a distinct population of glial precursor cells into oligodendrocytes. Dev Biol. 237(1):232-43

Morell P, Barrett CV, Mason JL, Toews AD, Hostettler JD, Knapp GW, Matsushima GK. (1998) Gene expression in brain during cuprizone-induced demyelination and remyelination. Mol Cell Neurosci. 12(4-5): p. 220-7.

Morest DK, Silver J. (2003) Precursors of neurons, neuroglia, and ependymal cells in the CNS: what are they? Where are they from? How do they get where they are going? Glia. 43(1):6-18.

Nait-Oumesmar B, Picard-Riéra N, Kerninon C, Baron-Van Evercooren A. (2008) The role of SVZ-derived neural precursors in demyelinating diseases: from animal models to multiple sclerosis. J Neurol Sci. 265(1-2):26-31.

Nave KA, Trapp BD. (2008) Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci. 31:535-61. Review.

Nery S, Wichterle H, Fishell G. (2001) Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. Development 128:527 – 540.

Nicolay D, Doucette R, and Nazarali A. (2004) Early stages of oligodendrocyte development in the embryonic murine spinal cord proceed normally in the absence of Hoxa2. Glia, 48(1): p. 14-26.

Nicolay DJ, Doucette JR, and Nazarali AJ. (2004b) Hoxb4 in oligodendrogenesis. Cell Mol Neurobiol, 24(3): p. 357-66.

Nicolay DJ, Doucette JR, Nazarali AJ. (2007) Transcriptional control of oligodendrogenesis. Glia. 55(13):1287-99. Review.

Nielsen JA, Berndt J, Hudson LD, Armstrong RC. (2004) Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells.

Mol Cell Neurosci 25:111 – 123.

Noble M. (2004). The possible role of myelin destruction as a precipitating event in Alzheimer's disease. Neurobiology of aging 25(1): 25-31.

Novitch BG, Chen AI, Jessell TM. (2001) Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron. 31(5):773-89.

O'Brien JS, Sampson. EL. (1965) Lipid composition of the normal human brain: gray matter, white matter, and myelin. J. Lipid Res. 6 537–544.

Ogata T, Yamamoto S, Nakamura K, Tanaka S. (2006) Signaling axis in schwann cell proliferation and differentiation. Mol Neurobiol. 33(1):51-62.

Olson JK, Miller SD. (2009) The innate immune response affects the development of the autoimmune response in Theiler's virus-induced demyelinating disease. J Immunol. 182(9):5712-22.

Ono K, Takebayashi H, Ikenaka K. (2009) Olig2 transcription factor in the developing and injured forebrain; cell lineage and glial development. Mol Cells. 27(4):397-401.

Ono K, Yasui Y, Rutishauser U, Miller RH. (1997) Focal ventricular origin and migration of oligodendrocyte precursors into the chick optic nerve. Neuron. 19(2):283-92.

Ono K, Takebayashi H, Ikeda K, Furusho M, Nishizawa T, Watanabe K, Ikenaka K. (2008) Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium. Dev Biol. 320(2):456-68.

Rakic P. (1995) Radial cells: scaffolding for brain construction. In: Neuroglia, edited by Kettenmann H and Ransom BR. Oxford, UK: Oxford Univ. Press, p. 746–762.

Rivers LM, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nature Neurosci 11:1392-1401.

Rodriguez M, Leibowitz JL, Powell HC, Lampert PW. (1983) Neonatal infection with the Daniels strain of Theiler's murine encephalomyelitis virus. Lab Invest. 49(6):672-9.

Park H-C, Appel B. (2003) Delta-Notch signaling regulates oligodendrocyte specification. Development 130:3747–3755.

Pekny M, Wilhelmsson U, Bogestål YR, Pekna M. (2007) The role of astrocytes and complement system in neural plasticity. Int Rev Neurobiol. 82:95-111.

Petters A, PalayS, Webster H de F. (1991) The fine sturcutre of the nervous system. Oxford, UK: Oxford Univ. Press.

Pfeiffer SE, Warrington AE, Bansal R. (1993) The oligodendrocyte and its many cellular processes. Trends Cell Biol. 3(6):191-7.

Pfrieger FW, Barres BA. (1997) Synaptic efficacy enhanced by glial cells in vitro. Science. 277(5332):1684-7.

Pham-Dinh D, Birling MC, Roussel G, Dautigny A, Nussbaum JL. (1991) Proteolipid DM-20 predominates over PLP in peripheral nervous system. Neuroreport. 2(2):89-92.

Power J, Mayer-Pröschel M, Smith J, Noble M. (2002) Oligodendrocyte precursor cells from different brain regions express divergent properties consistent with the differing time courses of myelination in these regions. Dev Biol. 245(2):362-75.

Price J. (1994) Glial cell lineage and development. Curr Opin Neurobiol. 4(5):680-6. Review.

Price J, Thurlow L. (1988) Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. Development.;104(3):473-82.

Pringle NP, Richardson WD. (1993) A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. Development. 117(2):525-33.

Raff MC. (1989) Glial cell diversification in the rat optic nerve. Science. 243(4897):1450-5.

Raff MC, Williams BP, Miller RH. (1984) The in vitro differentiation of a bipotential glial progenitor cell. EMBO J. 3(8):1857-64.

Raine CS. (1997) The Norton Lecture: a review of the oligodendrocyte in the multiple sclerosis lesion. J Neuroimmunol. 77(2):135-52.

Rajasekharan S. (2008) Intracellular signaling mechanisms directing oligodendrocyte precursor cell migration. J Neurosci. 28(50):13365-7. Review.

Readhead C, Schneider A, Griffiths I, Nave KA. (1994) Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron. 12(3):583-95.

Redwine JM, Blinder KL, Armstrong RC. (1997) In situ expression of fibroblast growth factor receptors by oligodendrocyte progenitors and oligodendrocytes in adult mouse central nervous system. J Neurosci Res. 50(2):229-37.

Richardson WD, Smith HK, Sun T, Pringle NP, Hall A, Woodruff R. (2000) Oligodendrocyte lineage and the motor neuron connection. Glia. 29(2):136-42. Review.

Rosetti CM, Maggio B, Oliveira RG. (2008) The self-organization of lipids and proteins of myelin at the membrane interface. Molecular factors underlying the microheterogeneity of domain segregation. Biochim Biophys Acta. 1778(7-8):1665-75.

Pivneva TA. (2008) Microglia in normal condition and pathology. Fiziol Zh. 54(5):81-9. Review.

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. (2008) PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci. 11(12):1392-401.

Samanta J, Kessler JA. (2004) Interactions between ID, OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development 131:4131 – 4142.

Schachner M, Bartsch U. (2000) Multiple functions of the myelin-associated glycoprotein MAG (siglec-4a) in formation and maintenance of myelin. Glia. 29(2):154-65. Review.

Schnaar RL, Lopez PH (2009) Myelin-associated glycoprotein and its axonal receptors. J Neurosci Res. [Epub ahead of print].

Schultz C, Dehghani F, Hubbard GB, Thal DR, Struckhoff G, Braak E, Braak H. (2000) Filamentous tau pathology in nerve cells, astrocytes, and oligodendrocytes of aged baboons. J Neuropathol Exp Neurol. 59(1):39-52.

Segal D, Koschnick JR, Slegers LH, Hof PR. (2007) Oligodendrocyte pathophysiology: a new view of schizophrenia. Int J Neuropsychopharmacol. 10(4):503-11.

Selkoe DJ. (1999) Translating cell biology into therapeutic advances in Alzheimer's disease. Nature. 399(6738 Suppl):A23-31.

Semendeferi K, Lu A, Schenker N, Damasio H. (2002) Humans and great apes share a large frontal cortex. Nat Neurosci. 5(3):272-6.

Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P. (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci. 2008 Aug 24.

Setoguchi T, Kondo T. (2004) Nuclear export of OLIG2 in neural stem cells is essential for ciliary neurotrophic factor-induced astrocyte differentiation. J Cell Biol 166: 963-968.

Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P. (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci. 11(9):1024-34.

Shie FS, Nivison M, Hsu PC, Montine TJ. (2009) Modulation of microglial innate immunity in Alzheimer's disease by activation of peroxisome proliferator-activated receptor gamma. Curr Med Chem. 16(6):643-51. Review.

Sim FJ, Zhao C, Penderis, J, and Franklin RJ. (2002) The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. J Neurosci, 22(7): p. 2451-9.

Skihar V, Silva C, Chojnacki A, Dring A, Stallcup WB, Weiss S, Yong VW. (2009) Promoting oligodendrogenesis and myelin repair using the multiple sclerosis medication glatiramer acetate. Proc Natl Acad Sci U S A. 106(42):17992-7.

Sohn J, Natale J, Chew LJ, Belachew S, Cheng Y, Aguirre A, Lytle J, Nait-Oumesmar B, Kerninon C, Kanai-Azuma M, Kanai Y, Gallo V. (2006) Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. J Neurosci. 26(38):9722-35.

Soustelle L, Giangrande A. (2007) Glial differentiation and the Gcm pathway. Neuron Glia Biol. 3(1):5-16.

Soustelle L, Roy N, Ragone G, Giangrande A. (2008) Control of gcm RNA stability is necessary for proper glial cell fate acquisition. Mol Cell Neurosci. 37(4):657-62.

Spassky N, Olivier C, Perez-Villegas E, Goujet-Zalc C, Martinez S, Thomas J, Zalc B. (2000) Single or multiple oligodendroglial lineages: a controversy. Glia. 29(2):143-8.

Steinman, L. (2001). Multiple sclerosis: a two-stage disease. Nature immunology 2(9): 762-764.

Stidworthy MF, Genoud S, Suter U, Mantei N, and Franklin RJ. (2003) Quantifying the early stages of remyelination following cuprizone-induced demyelination. Brain Pathol, 13(3): p. 329-39.

Sugimoto Y, Taniguchi M, Yagi T, Akagi Y, Nojyo Y, Tamamaki N. (2001) Guidance of glial precursor cell migration by secreted cues in the developing optic nerve. Development.128(17):3321-30.

Sun T, Dong H, Wu L, Kane M, Rowitch DH, Stiles CD. (2003). Cross-repressive interaction of the Olig2 and Nkx2.2 transcription factors in developing neural tube associated with formation of a specific physical complex. J Neurosci 23:9547-9556.

Sun X, Wang X, Chen T, Li T, Cao K, Lu A, Chen Y, Sun D, Luo J, Fan J, Young W, Ren Y. (2010) Myelin activates FAK/Akt/NF-kappaB pathways and provokes CR3-dependent inflammatory response in murine system. PLoS One. 5(2):e9380.

Tallon-Baudry C, Bertrand O, Fischer C. (2001) Oscillatory synchrony between human extrastriate areas during visual short-term memory maintenance. J Neurosci. 21(20):RC177.

Tatsumi K, Takebayashi H, Manabe T, Tanaka KF, Makinodan M, Yamauchi T, Makinodan E, Matsuyoshi H, Okuda H, Ikenaka K, Wanaka A. (2008) Genetic fate mapping of Olig2 progenitors in the injured adult cerebral cortex reveals preferential differentiation into astrocytes. J Neurosci Res. 86(16):3494-502.

Tekki-Kessaris N, Woodruff R, Hall AC, Gaffield W, Kimura S, Stiles CD, Rowitch DH, Richardson WD. (2001) Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. Development.128(13):2545-54.

Tetzloff SU, Bizzozero OA. (1998) Palmitoylation of proteolipid protein from rat brain myelin using endogenously generated 18O-fatty acids. J Biol Chem. 273(1):279-85.

Tochitani, S., Hayashizaki, Y. (2008). Nkx2.2 antisense RNA overexpression enhanced oligodendrocytic differentiation. Biochem Biophys Res Commun 372:691-696.

Todorich B, Zhang X, Slagle-Webb B, Seaman WE, Connor JR. (2008) Tim-2 is the receptor for H-ferritin on oligodendrocytes. J Neurochem. 107(6):1495-505.

Thal DR, Rüb U, Orantes M, Braak H. (2002) Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 58(12):1791-800.

Timsit S, Martinez S, Allinquant B, Peyron F, Puelles L, Zalc B. (1995) Oligodendrocytes originate in a restricted zone of the embryonic ventral neural tube defined by DM-20 mRNA expression. J Neurosci. 15(2):1012-24.

Torkildsen O, Brunborg LA, Myhr KM, Bø. (2008) The cuprizone model for demyelination. Acta Neurol Scand Suppl. 188:72-6. Review.

Traugott U, Raine CS, and McFarlin DE. (1985) Acute experimental allergic encephalomyelitis in the mouse: immunopathology of the developing lesion. Cell Immunol, 91(1): p. 240-54.

Vallstedt A, Klos JM, Ericson J. (2005) Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. Neuron. 45(1):55-67.

Vallstedt A, Muhr J, Pattyn A, Pierani A, Mendelsohn M, Sander M, Jessell TM, Ericson J. (2001) Different levels of repressor activity assign redundant and specific roles to Nkx6 genes in motor neuron and interneuron specification. Neuron 31:743-755.

Venancel L, Stella N, Glowinski J, Giaume C. (1997) Mechanism involved in initiation and propagation of receptor-induced intercellular calcium signaling in cultured rat astrocytes. J Neurosci 17: 1981–1992.

Verkhratsky A, Butt A. (2007) Glial Neurobiology: a textbook. ISBN 978-0-470-01564-3

Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. (2001) Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. Neurobiol Aging. 22(4):547-53.

Vlkolinský R, Cairns N, Fountoulakis M, Lubec G. (2001) Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. Neurobiol Aging. 22(4):547-53.

Voineskos AN, de Luca V, Bulgin NL, van Adrichem Q, Shaikh S, Lang DJ, Honer WG, Kennedy. (2008) A family-based association study of the myelin-associated glycoprotein and 2',3'-cyclic nucleotide 3'-phosphodiesterase genes with schizophrenia. Psychiatr Genet. 18(3):143-6.

Walsh MJ, Murray JM.(1998) Dual implication of 2',3'-cyclic nucleotide 3' phosphodiesterase as major autoantigen and C3 complement-binding protein in the pathogenesis of multiple sclerosis. J Clin Invest. 101(9):1923-31.

Wang C, Gold BG, Kaler LJ, Yu X, Afentoulis ME, Burrows GG, Vandenbark AA, Bourdette DN, Offner H. (2006) Antigen-specific therapy promotes repair of myelin and axonal damage in established EAE. J Neurochem. 98(6):1817-27.

Wang DD, Bordey A. (2008) The astrocyte odyssey. Prog Neurobiol. 86(4):342-67.

Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA. (1998) Notch receptor activation inhibits oligodendrocyte differentiation. Neuron. 21:63 – 75.

Wang S, Sdrulla A, Johnson JE, Yokota Y, Barres BA. (2001) A role for the helix-loop-helix protein Id2 in the control of oligodendrocyte development. Neuron 29:603 – 614.

Warf BC, Fok-Seang I, Miller RH. (1991) Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. J Neurosci. 11(8):2477-88.

Watanabe M, Hadzic T, Nishiyama A. (2004). Transient upregulation of Nkx2.2 expression in oligodendrocyte lineage cells during remyelination. Glia 46:311-322

Wei Q, Miskimins WK, Miskimins R. (2005). Stage-specific expression of myelin basic protein in oligodendrocytes involves Nkx2.2-mediated repression that is relieved by the Sp1 transcription factor. J Biol Chem 280:16284-16294.

Wolburg H, Noell S, Wolburg-Buchholz K, Mack A, Fallier-Becker P. (2009) Agrin, aquaporin-4, and astrocyte polarity as an important feature of the blood-brain barrier. Neuroscientist. 15(2):180-93. Review.

Wolswijk G, and Noble, M(1989) Identification of an adult-specific glial progenitor cell. Development 105(2): 387-400.

Woodhoo A, Sommer L. (2008) Development of the Schwann cell lineage: from the neural crest to the myelinated nerve. Glia. 56(14):1481-90. Review.

Woodruff RH, Tekki-Kessaris N, Stiles CD, Rowitch DH, Richardson WD. (2001) Oligodendrocyte development in the spinal cord and telencephalon: common themes and new perspectives. Int J Dev Neurosci. 19(4):379-85.

Yajima K. and Suzuki K. (1979) Demyelination and remyelination in the rat central nervous system following ethidium bromide injection. Lab Invest, 41(5): p. 385-92.

Yang Y, Liu Y, Wei P, Peng H, Winger R, Hussain RZ, Ben LH, Cravens PD, Gocke AR, Puttaparthi K, Racke MK, McTigue DM, Lovett-Racke AE. (2010) Silencing Nogo-A promotes functional recovery in demyelinating disease. Ann Neurol. 67(4):498-507.

Ye F, Chen Y, Hoang T, Montgomery RL, Zhao XH, Bu H, Hu T, Taketo MM, van Es JH, Clevers H, Hsieh J, Bassel-Duby R, Olson EN, Lu QR. (2009) HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. Nat Neurosci. 2009 Jul;12(7):829-38. Epub 2009 Jun 7.

Ye P, Hu Q, Liu H, Yan Y, D'ercole AJ. (2010) Beta-catenin mediates insulin-like growth factor-I actions to promote cyclin D1 mRNA expression, cell proliferation and survival in oligodendroglial cultures. Glia. 58(9):1031-41.

Yin X, Crawford TO, Griffin JW, Tu P, Lee VM, Li C, Roder J, Trapp BD. (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. J Neurosci. 18(6):1953-62.

Young KM, Fogarty M, Kessaris N, Richardson WD. (2007) Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. J Neurosci. 27(31):8286-96.

Zeng S, Jung P. (2008) Simulation analysis of intermodal sodium channel function. Phys Rev E Stat Nonlin Soft Matter Phys. 78(6 Pt 1):061916.

Zhang SC. (2001) Defining glial cells during CNS development. Nat Rev Neurosci. 2(11):840-3.

Zhao C, Fancy SP, Kotter MR, Li WW, Franklin RJ. (2005) Mechanisms of CNS remyelination--the key to therapeutic advances. J Neurol Sci. 233(1-2):87-91. Review.

Zhao C, Fancy SP, ffrench-Constant C, Franklin RJ. (2008) Osteopontin is extensively expressed by macrophages following CNS demyelination but has a redundant role in remyelination. Neurobiol Dis. 31(2):209-17.

Zhao JW, Raha-Chowdhury R, Fawcett JW, Watts C. (2009) Astrocytes and oligodendrocytes can be generated from NG2+ progenitors after acute brain injury: intracellular localization of oligodendrocyte transcription factor 2 is associated with their fate choice. Eur J Neurosci. 29(9):1853-69.

Zhou Q, Choi G, Anderson DJ. (2001) The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2. Neuron 31:791–807.

Zhou Q, Wang S, Anderson DJ. (2000) Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron 25:331–343.

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